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ULTRAVIOLET INDUCED DNA DAMAGE AND HEREDITARY SKIN CANCER

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Introduction

Human skin cells are almost constantly exposed to the UV radiation contained in sunlight that causes alterations in the cells' genetic material - DNA. Since damage to DNA may be harmful or lethal, it is no surprise that cells possess the ability to repair DNA. Research on mammalian systems including human cells has suggested that damage to DNA plays a causal role in carcinogenesis. UV light (254 nm) has been used to quantitate the DNA repair capacity of both procaryotes and eucaryotes. UV light induces a variety of lesions in DNA, however, the major product is the cyclobutyl pyrimidine dimer. A variety of effects are attributed to dimer presence in DNA. Among these are inactivation of cellular processes (particularly DNA synthesis), cell killing and mutation. The importance of dimers as lesions in cells is best illustrated by studies with cells derived from patients with a genetic disease. Cells from individuals who suffer from the disease xeroderma pigmentosm (XP) are both senstitive to UV-induced killing and mutation as well as defective in some aspect of the repair of DNA. This presentation will focus first on our work with pyrimidine dimers in the DNA of human cells and our current knowledge of induction and excision of this lesion. Secondly, on a UV induced photosensitive lesion whose rate of induction by 254 nm UV is roughly an order of magnitude lower than that of pyrimidine dimers, is detectable by 313 nm sensitivity (unlike pyrimidine dimers) and whose kinetic behavior after induction differs markedly among normal human cells, classical (XP) cells and XP variants.

Induction of dimers in DNA

UV light induces dimer formation between adjacent pyrimidines in DNA, giving rise to thymine-thymine, thymine-cytosine, and cytosine-cytosine (T<>T, T<>C, C<>C) dimers. About 90% of the damage induced in DNA by ultraviolet light occurs with the formation of these types of dimers. Using the procedures of two-dimensional paper chromatography, and an assay of UV-endonuclease-sensitive sites (ESS), dimers may be quantitated. The percent thymine in dimers increases with increase in dose of 254nm light. In addition to ascertaining the total number of dimers induced in DNA by various wavelengths of UV light, we determined the relative proportions of the three pyrimidine dimer types. Labeling of both thymidine and cytosine in human cells using [^{14}C]uridine as a precursor allows quantitation of all three dimers by using two-dimensional paper chromatography. After 50 Jm^{-2} of 254 nm light, the ratio of C<>C, C<>T, T<>T in human cellular DNA is 10:24:66. Due to the light absorption characteristics of cytosine, not only are fewer C<>C dimers produced; but, in addition, their incidence levels off more at lower doses than does that of the other forms. For example, while thymine-containing dimers may be induced to a level of about ten percent of the total pyrimidines, the C<>C dimer will make up only 0.05 percent of the total. Cytosine-containing dimers, however, make up a large portion of the total dimers when cells are irradiated with low biologically relevant doses of the wavelengths contained in sunlight. Since essentially no 254 nm radiation reaches the earth's surface, it was desirable to extend our observations to include studies with a source more closely approximating the wavelengths of natural solar light. We have conducted studies using a Westinghouse FS40 sunlamp that produces mostly near-UV but some

wavelength in the far-UV as well. The spectral irradiance of the cellulose acetate (Kodacel) filtered lamps closely simulates sunlight. A low dose of filtered FS40 light produces dimers at a ratio of 20:40:40 (C<>C, C<>T, T<>T) in human cells. The number and kinds of pyrimidine dimers induced in DNA by UV light are related to the DNA base ratio, the wavelength of UV light and the total dose. In human cells after low (biological) doses of 254 nm light, the thymine-containing dimers comprise about 75% of the total dimers - after "sunlight" the number is reduced to 60%. Whether or not this difference in the kinds of dimers seen at different wavelengths is biologically significant is at present unclear. If cells are exposed to longer UV wavelengths, much higher doses are needed to create the same number of dimers in the DNA. Increasing the wavelength from 254 to 313 nm requires a 2000-fold increase in dose to produce one million dimers per cell. Human cells in culture exposed to 100 min of sunlight results in the production of about one million dimers per cell- about the same number of dimers as produced by 10 Jm^{-2} of 254 light. However, it is estimated that only about ten percent of the wavelengths around 300 nm would penetrate the skin to the sensitive basal layers so that one could conclude that a biologically significant dose of 254 nm radiation might be in the neighborhood of 1-5 Jm^{-2}.

The number of UV-induced pyrimidine dimers excised from the DNA of human cells.

Pyrimidine dimers (lesions) are known to cause a variety of detrimental effects in cellular metabolic activities. The most readily apparent effect is on the synthesis of DNA. Pyrimidine dimers are blocks to DNA synthesis

and the cell has to devise a means of overcoming or bypassing this block. Most of DNA damage is repaired by a process called excision repair. Several enzymes act in a concerted fashion to remove damaged bases and replace them with the correct base. Human cells in culture are able to excise about one million dimers over a twenty-four hour period. The capacity derived from initial rates appears to indicate that these cells are able to excise about 10^5 dimers per hour. We have performed experiments relating the number of dimers excised with the amount of dimers introduced into the DNA by UV light of 254 nm. The results are shown in Table I. At doses of UV-light 20-60 Jm^{-2} the number of dimers excised reaches a maximum of around one million per cell. Doses higher than 60 Jm^{-2} seem to inhibit dimer removal. We analyzed for dimer content by two-dimensional paper chromatography of acid hydrolysates of the DNA, and by determining the number of enzyme- sensitive sites (ESS) present in the DNA. Both methods measure the amount of pyrimidine dimers in the DNA of UV-irradiated cells. At this time, we do not know the reason for the inhibition of dimer excision at doses greater than 60 Jm^{-2}. It is apparent from several lines of evidence that excision repair is a well coordinated multienzyme process involving a number of steps leading to the replacement of damaged nucleotides. We observe that breaks occur in the DNA at high doses, and this may well reflect the uncoupling of the steps of excision repair.

Excision of dimers from the DNA of cells exposed to simulated sunlight (FS40 Sunlamp)

Far-UV (254 nm) does not entirely simulate a biological system with respect to the effective wavelengths of ultraviolet light encountered in

sunlight. Therefore, it is of some interest to evaluate dimer removal following irradiation simulating a "natural" source. If one uses an FS40 sunlamp filtered with cellulose acetate to screen out wavelengths below 290 nm and the appropriate dose is given to human cells, dimers may be easily quantitated and the kinetics of their removal determined. Our results (Fig 1) indicate that the relationship between dose and the amount of dimers removed in 24 hrs is the same for both 254 nm irradiation and FS40 irradiation. The results using "sunlight" wavelengths suggest no excision repair sensitivity relative to 254 nm. Thus, the source of the insult to the cells is not an important factor in determining rate of dimer removal. If and when other photoproducts are formed by wavelengths between 290 and 400 nm (e.g., thymine glycols, pyrimidine-pyrimidone (6-4) photoproduct) the possible repair of these photoproducts does not interfere with the excision of pyrimidine dimers.

A New, Non-Dimer, Photosensitive DNA Lesion

Ultraviolet light causes a variety of alterations in the DNA of human cells. As noted before, the most prevalent lesion formed is the pyrimidine dimer. Its formation and biological consequences is well documented. We know that other DNA damage may be formed in smaller amounts and of unknown biological importance. Recently, the formation, lethality, and mutagenic action of a minor cytosine - thymine UV-product has been studied (Haseltine, Cell 33:13-17, 1983). This TC (6-4) product is formed at one-tenth the rate of pyrimidine dimers, but may be responsible for a major biological effect.

More recently, we have observed another alteration in the DNA of cells irradiated with far-UV light. When human tissue culture cells are given low doses of 254 nm light essentially no measurable single-strand breaks are formed in the DNA. When these lightly irradiated cells are exposed to near-UV light of 313 nm and sedimented in alkaline sucrose gradients, breaks appear in the DNA. These far-UV products senstitive to 313 nm light are formed in the DNA in increasing amounts with increasing doses of 254 nm light.

Twenty Jm^{-2} of 254 nm light produces about 2 breaks per 10^9 dalton DNA when irradiated with high doses (1.2×10^5 J/m^{-2}) of 313 nm light in all human cells tested. In normal human fibroblasts the 313 - sensitive - lesion disappears from the DNA during five hours of incubation in growth media (Table II). As noted before, normal human cells are able to excise pyrimidine dimers from their DNA. In cells derived from patients with the genetic disease, XP, other patterns of dealing with this product are observed. In XP cells most deficient in excision repair of pyrimdine dimers (XPA), the number of these products actually increases during a five hour period of incubation. In cells derived from XP patients proficient in dimer excision (XP-variant) the product remains in the DNA even after 20 hours of incubation. It should be noted that since these assays are done on alkaline sucrose gradients, we may be measuring the formation of alkaline sensitive sites rather than actual strand breaks occurring in the cells with UV irradiation. In either case, the breaks seen are a measurement of the UV-induced DNA lesion. The XPA mutation is inherited as a mendelian autosomal recessive. According to the one gene-one enzyme hypothesis, this indicates that there is a single molecular defect in XPA

patients that distinguishes them from normal individuals. From a large body of experimental evidence, that difference would seem to be a defect in the excision repair system. From this reasoning we suggest the difference seen in normal cells vs. XPA in the above experiments is due to the ability of normal cells to perform excision repair and the inability or XPA cells to do this. However, it is well known that XP variant cells have normal excision repair. Why then do they not excise the 313nm sensitive lesion as do the normal cells? We suggest this is because the lesion must first be modified to an intermediate that is a substrate for the excision repair system and that in XP variant cells this modification does not occur. The modified intermediate may be more sensitive to 313nm light than the original lesion and this could explain its apparent increase with time in the XPA cells. This explanation seems to fit the observed experimental data we have at the present time.

Summary

Clearly, cells from normal individuals possess the ability to repair a variety of damage to DNA. Numerous studies indicate that defects in DNA repair may increase an individual's susceptibility to cancer. It is hoped that continued studies of the exact structural changes produced in the DNA by environmental insults, and the correlation of specific DNA changes with particular cellular events, such as DNA repair, will lead to a better understanding of cell-killing, mutagenesis and carcinogenesis.

Figure Legends

Figure 1. The percent dimers removed in 24 hr from human cellular DNA following either various doses of 254nm light or sunlight - simulating-sunlamps irradiation.

Table I

Pyrimidine Dimer Excision Repair in Cultured Human Fibroblasts During 24 Hours After UV-Irradiation

Jm^{-2} of 245 nm UV	Dimers excised/cell (times 10^6)	ESS excised/ 10^8 d DNA
10	0.72	15
20	0.96	28
40	1.20	28
60	1.10	20
80	0.72	18

Table II

Observed Strand Breaks/10^9 Daltons DNA After Photolysis

Hours after UV	0	1	3	5	20
XP A	1.8	2.2	2.4	3.6	3.5
Normal Cells	1.8	1.6	0.4	0.2	0.1
XP Var. (WOMEC)	1.9	--	--	1.8	1.3
XP Var. (PHEY)	1.4	--	--	1.4	1.4

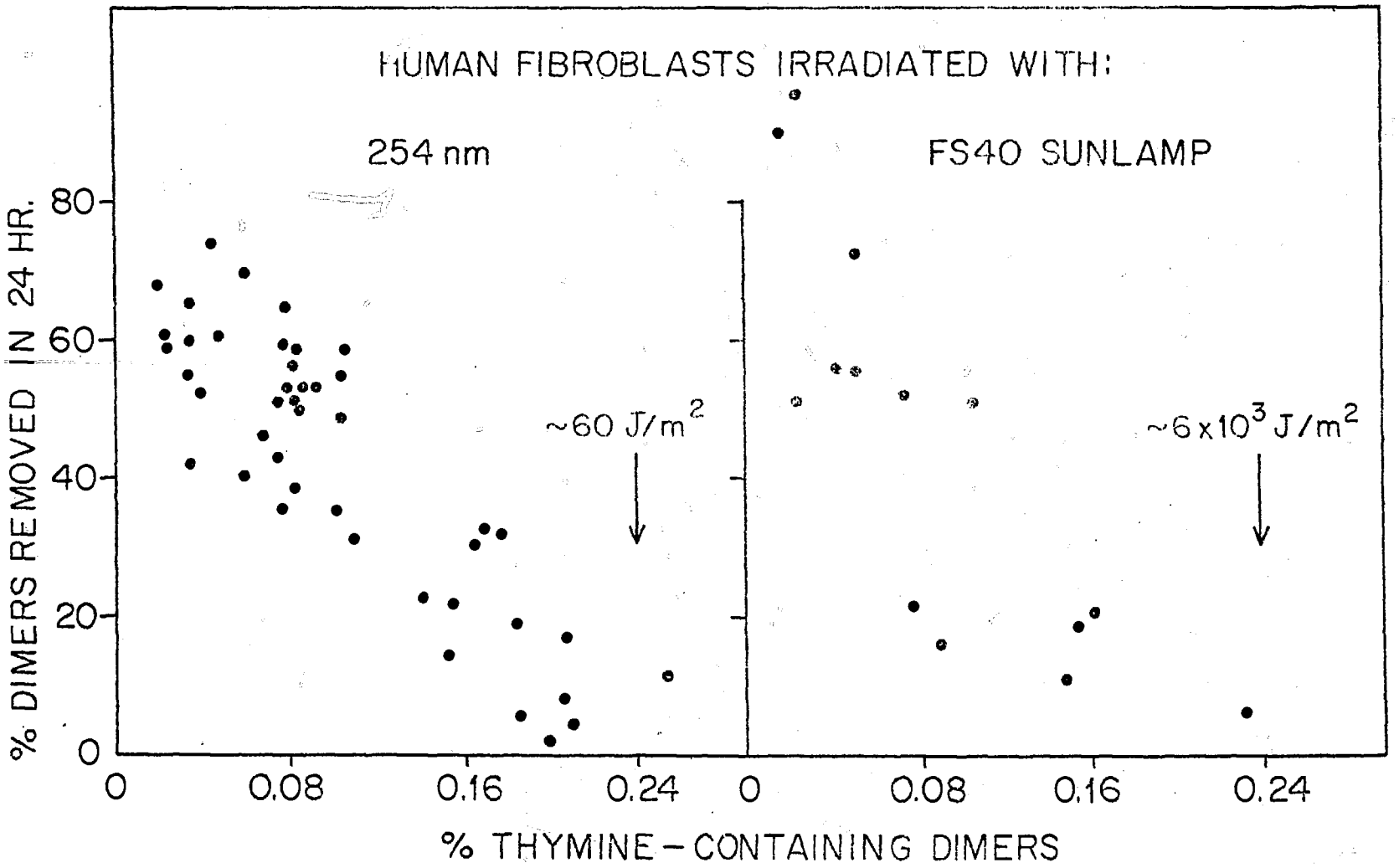
HUMAN FIBROBLASTS IRRADIATED WITH:
254 nm
FS40 SUNLAMP
~60 J/m²
~6x10³ J/m²
% DIMERS REMOVED IN 24 HR.
0
20
40
60
80
0
0.08
0.16
0.24
0
0.08
0.16
0.24
% THYMINE-CONTAINING DIMERS

Figure 1