EVIDENCE FOR EXCISION OF ULTRAVIOLET-INDUCED PYRIMIDINE DIMERS FROM THE DNA OF HUMAN CELLS IN VITRO

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ABSTRACT Within 12–24 hr after human cells were irradiated with ultraviolet light, approximately 50% of the ultraviolet-induced pyrimidine dimers were lost from the DNA. Pyrimidine dimers were found in the TCA-soluble fraction of ultraviolet-irradiated cells at 24 hr. Excess thymidine, caffeine, or hydroxyurea had no effect on the loss of pyrimidine dimers from the DNA of ultraviolet-irradiated cells.

INTRODUCTION

The excision of ultraviolet (UV)-induced pyrimidine dimers from the DNA of certain bacteria is a well-established phenomenon (1). In a number of studies, pyrimidine dimers have been induced in Chinese hamster (2–4) and mouse "L" cells (5, 6). However, in these studies no evidence was found for the loss or "excision" of dimers from the DNA subsequent to UV-irradiation.

In this report, evidence is presented that human cells in vitro differ markedly from the rodent cells previously studied in that they consistently lose a significant proportion of UV-induced pyrimidine dimers from their DNA. It is possible that this process is analogous to the dark repair phenomenon in UV-resistant bacteria. A preliminary report of this work has appeared elsewhere (7).

EXPERIMENTAL PROCEDURES

Cell Lines and Medium

The recovered amnion (RA) cell line (8) derived from human amnion, RAX10 [an RA subline derived from survivors of 1000 R of X-rays[1]], HeLa (9), and the diploid cell strain WI-38 (10) were used in these experiments. Cells were grown in monolayer in Eagle's minimal essential medium (11) with 10% calf serum at 37°C in a humidified atmosphere containing 2% CO_2.

[1]Regan, J. D. 1966. Unpublished experiments.

Irradiation and Dimer Analysis

Cells were grown in 40 mm quartz dishes and irradiated from below with 2650 A radiation from a Hilger monochromator (Hilger and Watts, Ltd., London, England) equipped with a 500 watt high pressure mercury lamp. Cells were labeled with tritiated thymidine (SA 1.9 c/mmole) at 1 or 2 μc/ml final concentration for 6–24 hr; radioactive medium was replaced with nonradioactive medium 2 hr before irradiation. At various times after irradiation, separate monolayers were washed once in balanced salt solution to remove dead cells, scraped from the dishes with a rubber policeman, and then collected by centrifugation. The DNA was precipitated with 5% cold TCA, hydrolyzed, and then analyzed for dimers by two-dimensional paper radiochromatography according to the method of Setlow and Carrier (1). TCA-soluble cell fractions were similarly analyzed.

RESULTS

Fig. 1 shows the relation between UV dose and the induction of pyrimidine dimers. The doses used in these studies were chosen because it was considered desirable to work in the sublethal range of these cells. After postirradiation periods of up to 72 hr there were still sufficient attached cells on which to perform the dimer analyses at these doses. Dimer analysis was thus performed on cells alive by the criterion of adherence. Not all such cells were necessarily capable of sustained proliferation.

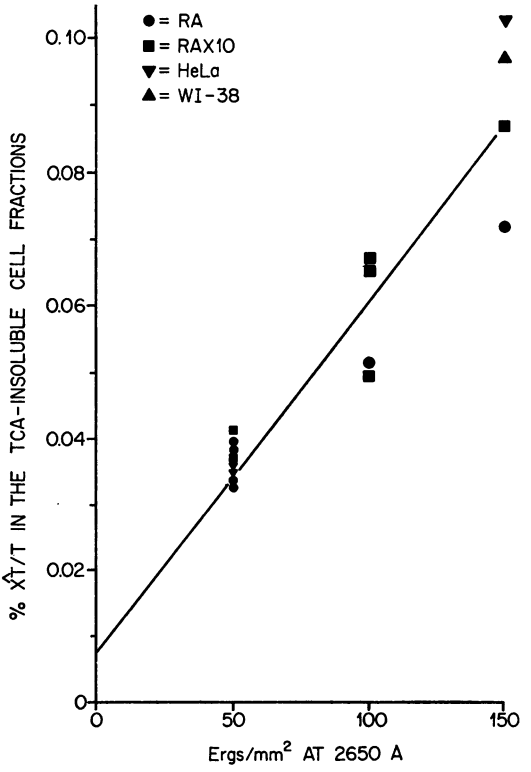

FIGURE 1 Induction of pyrimidine dimers in human cells by UV-irradiation. $\widehat{XT}$ denotes the total photoproduct radioactivity in the chromatograph region occupied by $\widehat{UT}$ and $\widehat{TT}$ (see text). Radioactivity of unirradiated cells in the dimer region was 0.009%.

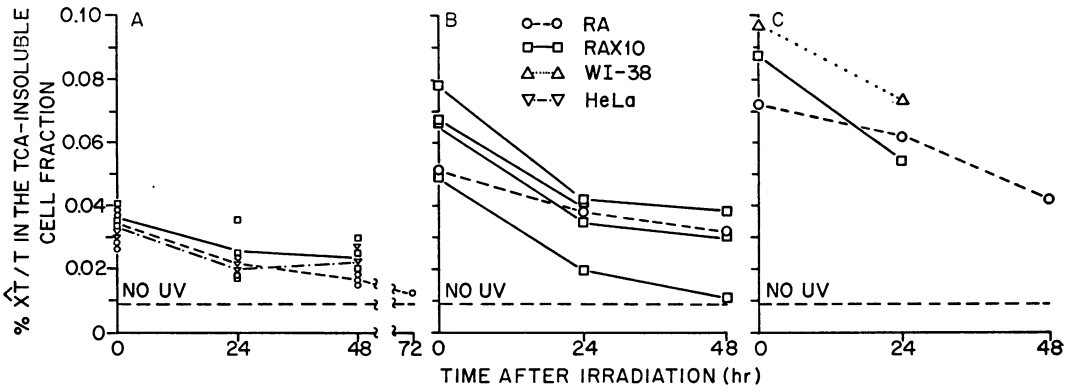

FIGURE 2 Loss of pyrimidine dimers from the TCA-insoluble cell fraction. A = 50 ergs/mm²; B = 100 ergs/mm²; C = 150 ergs/mm². Points shown in 2 A are experimental values. Lines connect mean values for each cell line. In 2 B and 2 C, lines connect values of individual experiments.

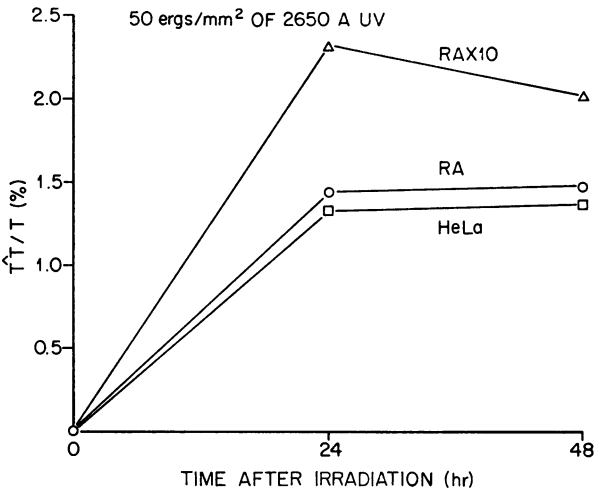

FIGURE 3 Appearance of pyrimidine dimers in the TCA-soluble cell fraction.

Precision of dimer analysis was, of course, somewhat reduced at such low radiation doses.

Figs. 2 *A*, *B*, and *C* show the loss of dimers from the TCA-insoluble fractions of the cells at 50, 100, and 150 ergs/mm², respectively. Although there was considerable variability, there was a consistent loss of dimers during the first 24 hr in all the cells studied. A significant fraction of the dimers appeared in the TCA-soluble cell fraction 24 hr after irradiation (Fig. 3), but no further increase was found at 48 hr.

Fig. 4 shows the results of experiments performed to determine the amount of radioactivity released into the medium by RAX10 cells during these experiments. Such release was caused by cell lysis due to incorporation of radioactivity and to

UV-irradiation. At 24 hr there is little difference between cells exposed to either 0, 100, or 150 ergs/mm² of irradiation and no difference at all between cells exposed to 0 or 50 ergs/mm², although at 48 and 72 hr considerable radioactivity from both irradiated and unirradiated samples appeared in the medium. Thus if "excision" is due only to DNA breakdown, cell lysis, and selective reincorporation of thymine but not dimer, then little radioactivity is available in the medium for such reincorporation at 24 hr, the time when the most excision is seen.

We wished to determine whether the removal dimers could be detected in purified DNA preparations (21) of UV-irradiated human cells; we also wished to separate homodimers ($\widehat{TT}$) from heterodimers ($\widehat{UT}$) in order to determine their relative con-

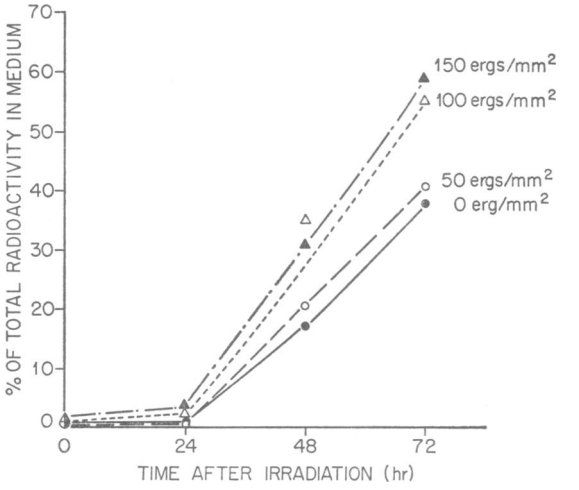

FIGURE 4 Appearance of radioactivity in the medium after UV-irradiation of ^{3}HT-labeled cells. Cells were collected by scraping and centrifugation. 100 μl aliquots of supernatant medium were counted in a scintillation counter by means of dioxane-based scintillator. Pellets were dissolved in 1 ml of 0.5 N NaOH, and 100 μl aliquots were similarly assayed for radioactivity.

tribution to total dimer loss. Table I shows the result of these experiments. $\widehat{UT}$ (induced as cytosine-thymine dimer but deaminated during acid hydrolysis and hence assayed as uracil-thymine) was induced at about one-third the amount of $\widehat{TT}$ and was lost at roughly the same percentage in 24 hr. A portion of the purified DNA preparation was passed over Sephadex G-100 to determine if there were any dimers lost as parts of a TCA-insoluble oligonucleotide. The results of that analysis were negative.

Results of experiments conducted to determine the time course of dimer loss during the first 24 hr are shown in Fig. 5. The most significant loss occurred in the first 12 hr. HeLa, however, exhibited a sharp decrease in dimers in 3 hr. The HeLa experiment in this case was done by irradiating the cells in monolayer, then placing them into swirl suspension cultures (12) so that identical aliquots could be drawn from the same culture, rather than employing parallel but separate cultures as in previous experiments. The sharp decrease seen in the HeLa experiment could be an artifact induced by transfer or different growth conditions.

TABLE I

LOSS OF HOMODIMER ($\widehat{TT}$) AND HETERODIMER ($\widehat{UT}$) FROM THE DNA OF RAX10 CELLS AFTER UV-IRRADIATION (DOSE = 100 ergs/mm²)

Per cent of thymine as $\widehat{TT}$, $\widehat{UT}$, or total ($\widehat{XT}$) at 0 and 24 hr after irradiation

Dimer	0 hr	24 hr
$\widehat{TT}$	0.044	0.028
$\widehat{UT}$	0.016	0.007
$\widehat{XT}$	0.060	0.035

DNA was purified by the method of Marmur (21). The dimers were separated by paper chromotography, with *n*-butanol–acetic acid–water (80:12:30) being used as the solvent, followed by *n*-butanol–water (86:14) in the second dimension.

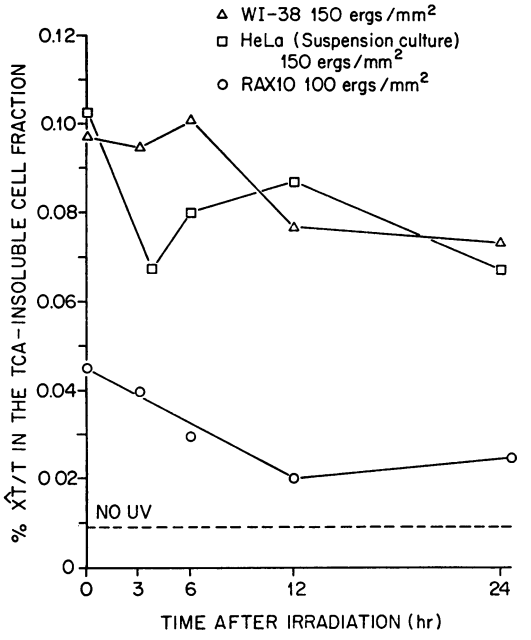

FIGURE 5 Time course of dimer loss in three human cell lines. HeLa was labeled and irradiated in monolayer and then placed in suspension culture. WI-38 and RAX10 were maintained in monolayer throughout the experiment.

The data presented here are based on the ratio of radioactivity in thymine-containing dimers to radioactivity in thymine alone. It was possible that there was extensive intracellular breakdown of DNA and that radioactive thymine, but not thymine-containing dimer, was being selectively reincorporated by the cells. Since this could produce an apparent but spurious dimer "excision", we examined the

effect of excess nonradioactive thymidine on dimer loss. The results are shown in Table II, where it can be seen that thymidine at 10^{-5} M had no effect on dimer loss.

Caffeine at relatively high concentrations is known to inhibit dimer excision in bacteria (13); caffeine can also inhibit colony formation in mouse cells after UV-irradiation (14). In our experiments, caffeine at a concentration of 9×10^{-3} M had no effect on dimer loss (Table II).

It has been shown that hydroxyurea at a concentration of 2.5×10^{-3} M reduces DNA synthesis in human cells in vitro to a level which is 2% that of control cultures (15). We therefore used this agent to determine if inhibition of DNA synthesis to this level would affect dimer removal. Dimer loss still occurred in the presence of hydroxyurea (Table II).

TABLE II

EFFECT OF SEVERAL AGENTS ON LOSS OF DIMERS FROM UV-IRRADIATED RAX10 CELLS (DOSE = 150 ergs/mm²)

Per cent of thymine as dimer

	0 hr	24 hr
	%	%
Control	0.082	0.047
Thymidine (1×10^{-5} M)	0.101	0.048
Caffeine (9×10^{-3} M)	0.078	0.039
Hydroxyurea (2.5×10^{-3} M)	0.066	0.038
Average	0.092	0.043

All agents are final concentrations in the medium and were added to cultures immediately after irradiation. Values are not corrected for radioactivity in the dimer region of unirradiated cells (0.009%).

DISCUSSION

The experiments reported here indicate that UV-induced pyrimidine dimers are consistently lost from the DNA of human cells in vitro with time after irradiation. Human cells appear to differ markedly from the other mammalian cells that have been examined in this respect (2–6). Cleaver (16) observed that human cells differed from mouse cells in that UV-irradiation stimulated a low level of non-"S" tritiated thymidine incorporation in human cells but not in mouse cells. In recent experiments Cleaver and Painter (17) presented evidence for "repair replication" in HeLa cells and showed that hydroxyurea, while inhibiting semiconservative replication, had no effect on repair replication. Djordjevic and Tolmach (18) recently confirmed UV-induced "unscheduled" DNA synthesis, in G_1 and G_2 HeLa cells. In their experiments 2.5×10^{-3} M hydroxyurea did not inhibit non-"S" synthesis.

Coyle and Strauss (19) observed that after UV-irradiation human cell DNA gradually became less sensitive to an endonuclease specific for pyrimidine dimers from *Micrococcus lysodeikticus*. Crathorn and Roberts (20) induced lesions in HeLa

cell DNA with alkylating agents and found that about 50% of the lesions were lost after 24 hr.

As is apparent from our data there is a loss of pyrimidine dimers from human cell DNA after UV-irradiation. However, we have been unable to achieve greater than 50–60% dimer removal. This fraction of dimers is lost within the first 12–24 hr. with reduced or no further dimer removal thereafter. We do not know how this 50–60% is distributed among surviving (i.e., adhering) cells or among DNA molecules in cells and we cannot as yet make any meaningful statement regarding the relationship of the phenomenon of dimer removal to survival in human cells.

The present data, together with the observations of other workers cited above, suggest that there are some similarities between the reaction of human cells to UV-irradiation and UV-repair mechanisms in bacteria. It is particularly remarkable that other mammalian cells examined have failed to show any indication of such a phenomenon and that human cells seem to be exceptional in this respect among cell types thus far studied.

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