Inhibition of messenger RNA accumulation but not translation in ultraviolet irradiated hepatoma cells

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Summary:

Irradiation by ultraviolet light at doses between 30 and 90 ergs/mm^2 increasingly inhibited the hydrocortisone but not the insulin induction of tyrosine aminotransferase activity in Reuber (H35) hepatoma cells in culture. Incorporation of leucine into cellular protein was only slightly inhibited in this dose range. Incorporation of uridine into RNA in the presence of low doses of actinomycin D (0.1 µg/ml) was increasingly inhibited by ultraviolet irradiation. Since cellular uptake of uridine into the acid soluble pool was not inhibited by ultraviolet irradiation, the inhibition of RNA labeling suggests inhibition of synthesis of heterogenous nuclear RNA by low doses of ultraviolet irradiation. "Unscheduled" DNA synthesis was initially stimulated 2 fold by 30 ergs/mm^2 irradiation and returned to control levels in 8 hours. Thus, ultraviolet irradiation in the low dose range causes DNA repair synthesis, inhibits transcription and apparently tyrosine aminotransferase messenger RNA accumulation while pre-existing cytoplasmic messenger appears not to be inactivated nor its translation inhibited.

Ultraviolet light is a mutagen and carcinogen. Ultraviolet induced biological damage is presumably related to formation of pyrimidine dimers in nucleic acid (1,2) and formation of DNA to protein cross links (3). In the present studies, initial effects of low doses of ultraviolet irradiation on "unscheduled" DNA synthesis, uridine incorporation into RNA, leucine incorporation into protein and hormonal induction of tyrosine aminotransferase were studied in cultured hepatoma cells. The doses of ultraviolet irradiation used (between 30 and 90 ergs/mm^2) are comparable with doses used in studies on DNA repair and mutation frequency and cell survival.

Methods and Materials

Reuber (H35) cells were grown as monolayers on 60 mm plates in 4 ml of Swim's S-77 medium which was modified to contain 4 mM glutamine and supplemented with 20% horse serum and 5% fetal calf serum. The gas phase was 5% CO_2 and 95% air. Cells were grown until each plate contained about 3 x 10^6

Table I - Effects of ultraviolet irradiation on the hydrocortisone induction of tyrosine aminotransferase.

Treatment	Units tyrosine aminotransferase	
Dose (ergs/mm^2)	Basal	Plus hydrocortisone
0	0.73 ± 0.02	4.71 ± 0.15
30	0.66 ± 0.06	3.96 ± 0.18
60	0.46 ± 0.02	2.26 ± 0.05
90	0.43 ± 0.02	1.48 ± 0.06

Cells were irradiated and then incubated for 6 hours with or without the addition of hydrocortisone (2×10^{-6} M). Cultures were harvested and assayed for tyrosine aminotransferase activity as described in Methods and Materials. Each value is the mean of 3 plates with the indicated standard deviation.

nuclei and 1.2 mg protein. At this point, cells were transferred to S-77 medium containing 4 mM glutamine but lacking serum. Experiments were conducted 18 hours after medium change. Inducing agents were dissolved in S-77 medium. For tyrosine aminotransferase assay, cells were harvested and a supernatant was prepared by freeze-thawing and low speed centrifugation as previously described (4). Tyrosine aminotransferase activity was determined by the method of Diamondstone (5). One unit of activity was defined as equal to formation of 1 µmole of p-hydroxyphenylpyruvic acid per hour per mg of supernatant protein at 37°C. Protein was determined by the method of Lowry (6). Incorporation of [^{3}H]-leucine into protein was determined by a filter disc method (7). RNA was determined by the method of Fleck and Munro (8), and radioactivity in RNA was determined as previously described (9). "Unscheduled" DNA synthesis was determined by a modification of the method of Trosko and Yager (10). Irradiation was performed with a 15 W germicidal lamp with output predominately at 254 nm. Before irradiation, the medium was removed from all plates. The appropriate plates were irradiated and the medium was added back to all plates.

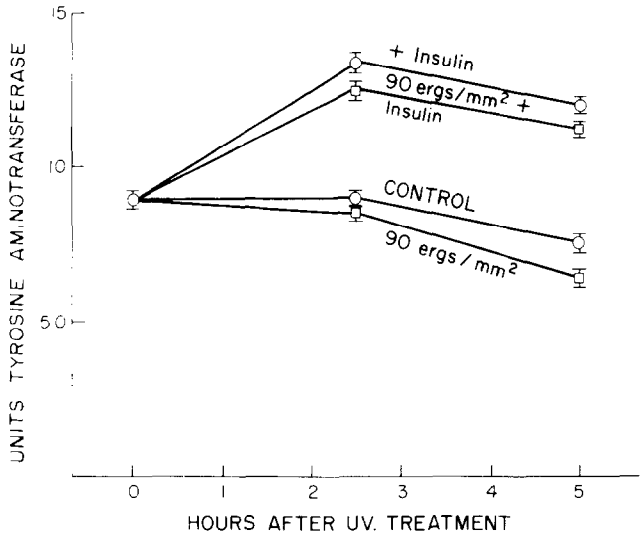

Fig. 1 Effect of ultraviolet irradiation (UV.) on insulin induction of tyrosine aminotransferase activity in hydrocortisone pre-induced cells. Hydrocortisone (2×10^{-6}M, final concentration) was added 18 hours prior to irradiation. After irradiation (90 ergs/mm^2), insulin (0.5 µg/ml) was added and cultures were harvested at 0, 2.5 and 5.0 hours after irradiation. Each point represents the average of 4 plates with range as indicated.

Results

Table I illustrates that increasing ultraviolet irradiation at doses between 30 and 90 ergs/mm^2 increasingly inhibited the hydrocortisone induction of tyrosine aminotransferase activity. Figure 1, however, demonstrates that ultraviolet irradiation at 90 ergs/mm^2 did not block the translational induction of tyrosine aminotransferase activity by insulin.

Figure 2 shows that ultraviolet irradiation in the 30 to 90 ergs/mm^2 dose range increasingly inhibited uridine incorporation into RNA in the presence of low doses of actinomycin D (0.1 µg/ml). Low doses of actinomycin D at this cell concentration selectively inhibit ribosomal precursor RNA synthesis in mammalian cells while permitting heterogenous nuclear RNA synthesis to continue at near normal rates (11). Cellular uptake of [^{3}H]-uridine into the acid soluble pool under these conditions was not inhibited by ultraviolet treatment (data not shown).

Table II - Effects of ultraviolet irradiation on ^{3}H-leucine incorporation into protein.

CPM ^{3}H-leucine into protein/μg protein		
Control	60 ergs/mm^2	90 ergs/mm^2
123 ± 8	119 ± 4	107 ± 5

Cells were irradiated and ^{3}H-leucine (1.2 μC/ml) was added to the S-77 medium. Cells were incubated 2 hours and then the medium was aspirated and the cultures were rinsed twice with 4 mls of isotonic saline. Cells were suspended in 2 ml isotonic saline with the aid of a rubber policeman. 0.1 ml aliquots were transferred to Whatman 3 MM filter discs and assayed for radioactivity in protein (7). Protein was determined on separate aliquots according to the method of Lowry (6). Each value represents the average of 4 culture dishes with the standard deviation given.

[^{3}H]-leucine incorporation into protein was only slightly inhibited initially by ultraviolet treatment (Table II).

Figure 3 illustrates the time course of DNA repair in H35 cells after ultraviolet irradiation at the dose of 30 ergs/mm^2. The initial 2 fold stimulation of "unscheduled" [^{3}H]-thymidine incorporation into DNA was considerably lower than seen in several other cell lines (10) under similar conditions suggesting that "excision" repair in this cell line may be limited. Further, incorporation returned to control levels within 8 hours indicating rapid cessation of ultraviolet light stimulated "excision" repair in these non-dividing hepatoma cells.

Discussion

Irradiation of cells with ultraviolet light leads to formation of thymidine dimers in DNA and considerable enhancement of the mutation frequency (1,2). Ultraviolet irradiation studies have proven useful in studies relating DNA damage and repair to mutation (1,2).

In bacteria, induction of β-galactosidase is inhibited by ultraviolet irradiation (1,12,13,14). This inhibition can be reversed by photoreactivation which causes monomerization of pyrimidine dimers (see 1). Further, studies

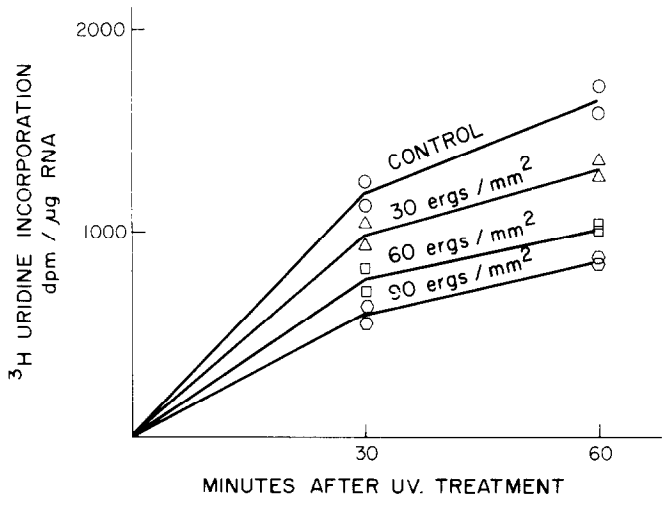

Fig. 2 Effects of ultraviolet (UV.) irradiation on incorporation of ^{3}H-uridine into RNA in cultures pretreated with low doses of actinomycin D. Actinomycin D (0.1 µg/ml) was added 30 minutes prior to irradiation. Cells were irradiated and ^{3}H-uridine was added to 2.5 µCi/ml (10^{-6}M final concentration). Cells were harvested at 30 and 60 minutes after irradiation and RNA and radioactivity in RNA were determined (8,9).

indicate that some of the newly synthesized messenger RNA for ß-galactosidase and for alkaline phosphatase in ultraviolet treated bacteria were translated to produce abnormal enzyme protein (13). Transcription of ultraviolet irradiated DNA by RNA polymerase is altered in vitro (15). Thus, effects of ultraviolet irradiation on transcription and translation appear largely due to formation of pyrimidine dimers in DNA (see 1,12,13).

Transcription of heterogenous nuclear RNA was inhibited in the Ehrlich ascites mammalian cell line by ultraviolet irradiation (16). Newly synthesized RNA chains were shortened in ultraviolet irradiated Erhlich cells suggesting premature release of unfinished RNA chains as a mechanism in this cell type (16). However, high doses of irradiation (0.5 to 1.0 x 10^3 ergs/mm^2) were used in these studies (16).

Ultraviolet irradiation in the 30 to 90 ergs/mm^2 dose range progressively inhibited the hydrocortisone but not the insulin induction of tyrosine

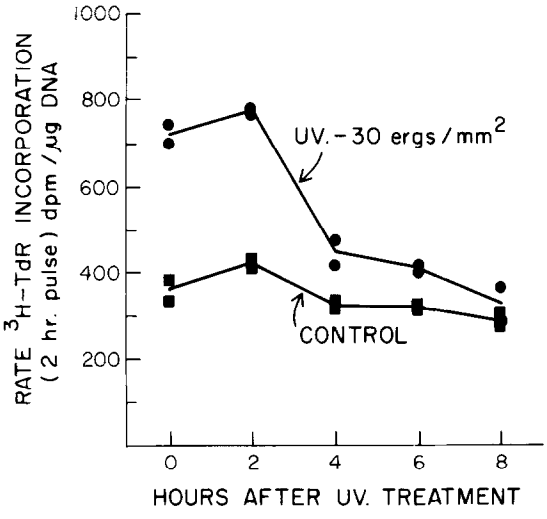

Fig. 3 Effects of ultraviolet light (UV.) on incorporation of ^{3}H-thymidine into DNA in the hydroxyurea pretreated cultures. The measurement of "unscheduled" DNA synthesis was modified from the procedure of Trosko and Yager (10). S77 medium rather than an arginine minus medium was used and hydroxyurea (10 mM) was added at 13 hours and again at 1 hour prior to irradiation. The long pretreatment with hydroxyurea was necessary to reduce thymidine incorporation to a low basal level. Cells were irradiated and at various times after irradiation pulsed for 2 hours with ^{3}H-thymidine (5 µCi/ml medium, 40 Ci/mmole). Incorporation into DNA and DNA content were determined as described elsewhere (10).

aminotransferase activity (Table I, Figure 1). Hydrocortisone appears to induce tyrosine aminotransferase activity via increased accumulation of newly transcribed tyrosine aminotransferase messenger RNA (see 4). Insulin induction, however, appears to result from increased translation of existing messenger RNA (17). Our RNA and protein incorporation studies suggested progressive inhibition of heterogenous nuclear RNA synthesis with only slight immediate inhibition of translation. These preliminary studies suggest that ultraviolet irradiation in sublethal doses inhibits gene transcription and functional tyrosine aminotransferase messenger RNA accumulation without greatly inactivating or inhibiting translation of pre-existing cytoplasmic messenger RNA.

The present results suggest that function of the tyrosine aminotransferase gene may be inhibited with only slight DNA damage. Ultraviolet (254 nm)

irradiation at 100 ergs/mm^2 of monolayers of Chinese hamster cells resulted in conversion of only 0.03% of thymidines to dimers in DNA (18). Since the precursor of mammalian messenger RNA is many times the size of the final molecule, a small amount of DNA damage may have large effects on functional messenger production. This contrasts with bacteria where RNA synthesis inhibition is quite insensitive to ultraviolet irradiation compared with colony formation (19). Inhibition of transcription is presumably due to inhibition of RNA polymerase at pyrimidine dimer sites although a role for ultraviolet induced DNA to protein cross links may also be important. Ultraviolet induced damage to DNA may vary with its chromosomal location; thus certain genes may be inactivated at lower doses when compared with other genes. Likewise, repair may be more rapid for some sites compared with others (20).

Since the working hypothesis is that inhibition of transcription in ultraviolet treated cells is related to formation of pyrimidine dimers in DNA and protein-DNA cross links, transcription should return to control levels when these lesions have been repaired. That Reuber hepatoma cells are capable of only limited DNA "excision" repair was demonstrated in figure 3. However, several other cell lines have been shown to be considerably more active in this type of DNA repair and therefore more suitable for correlation of transcriptional recovery with DNA repair (10). This correlative approach may aid in understanding the biological significance of different DNA repair mechanisms.

Acknowledgements:

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