

The Effect of UVA and UVB on DNA Synthesis and Unscheduled DNA Synthesis in Mouse Skin †

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= Abstract =The effects of ultraviolet light A (UVA) and ultraviolet light B (UVB) on DNA synthesis and unscheduled DNA synthesis (UDS) were studied in mouse skin by microautoradiography. The mice were exposed to 50mJ/cm² UVB by fluorescent sunlamp or 50J/cm² UVA by metal halide mercury lamp. Time course studies were performed immediately, 6 hours, 24 hours, and 48 hours after UVA and UVB exposure. There was no decreased number of heavily labeled cells (HLC) representing DNA synthesis immediately after UVA exposure and up to 48 hours postirradiation. However, immediately after and at 6 hours after UVA irradiation there was an increased number of sparsely labeled cells (SLC) representing UDS. Recovery was noticed 24 hours after irradiation and it was maintained after 48 hours postirradiation. These results clearly demonstrate that UVA induces considerable DNA damage and repair. DNA synthesis decreased immediately and at 6 hours and at 24 hours after UVB exposure. It recovered at 48 hours after UVB exposure. UDS increased immediately and at 6 hours after UVB exposure. Repair synthesis was completed at 24 hours after UVB exposure.

Key Words: UVA, UVB, Autoradiography, DNA synthesis, Unscheduled DNA synthesis

INTRODUCTION

Exposure of skin to ultraviolet light (UVL) has been shown to result in various biologic changes (Gange and Parrish 1983; Kochevar 1983). UVL irradiation on epidermal cells results in changes in DNA synthesis which can be detected by autoradiography using tritiated thymidine as a tracer to labeled nuclei. Heavily labeled cells (HLC) seen in the basal layer

on autoradiography represent the DNA synthesizing phase of the cell cycle (Epstein *et al.* 1968; Epstein *et al.* 1969).

UVL is a known carcinogen and mutagen. The effect of DNA repair on cutaneous carcinogenesis and mutagenesis has received much attention. UVL induced DNA repair can be detected by unscheduled DNA synthesis (UDS), which is known as sparsely labeled cells (SLC) characterized by sparse labeling of epidermal nuclei in autoradiography (Parrish *et al.* 1978; Sutherland *et al.* 1980; Ambrosio *et al.* 1981; Lewensohn and Ringbory 1985).

The presently used classification of UVL depicts three bands according to the UVL spectrum; UVA (320-400nm), UVB (290-320nm) and UVC (200-290nm). Most of the knowledge accumulated till now has been based on the

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effects of UVB. UVA had been thought to be biologically inert. However, recent studies on UV-induced skin change have reported increasing awareness of the fact that UVA is photobiologically active (Kaidbey and Kligman 1978; Greaves and Briffa 1981). Recent development of a potent and pure UVA device has led to some interesting studies concerning the nature of UVA (Mutzhas *et al.* 1981).

We have already studied the effect of UVB by high pressure mercury arc on DNA synthesis and UDS (Youn *et al.* 1989; Shin *et al.* 1990). In this study we used a fluorescent sunlamp as a UVB source which has its peak irradiance confined to UVB. As a UVA source a metal halide mercury lamp with a filter which cut off UVB and UVC, was used.

Our study was designed to determine the effect of UVA and UVB on DNA synthesis and UDS.

The effect of UVL was studied before and immediately after UVL exposure. We also checked the effect of UVL at 6hr, 24hr, and 48hr intervals after UVL exposure in order to observe changes according to the time course after UV irradiation.

MATERIALS AND METHODS

Materials

Experimental animals

Forty five 5 to 7 week old, 25-30gm female albino mice were used.

Light source

1) UVA : The UVA radiation source was SUPUVASUN 3000, a metal halide mercury lamp (Mutzhas Co., Germany). The UV radiation provided was potent UVA (330-460nm, peak 360nm). UVB, and UVC were excluded by filter. A UVASUN meter (Mutzhas Co., Germany) was used to measure the UV irradiance at skin level.

2) UVB : The UVB radiation source was a Waldmann UV 800 (Waldmann Co., Germany). The UV radiation provided was UVB (285-350nm, peak at 310-315nm). UV irradiance was measured with a Waldmann UV meter (Waldmann Co., Germany).

Radioactive tracer

Tritiated thymidine (TdR-³H NEN Co., USA) with a specific activity of 20Ci/mM was diluted with normal saline to 10 Ci/mM.

Methods

1) UVL irradiation

The backs of the mice were shaved with an electric clipper, Styler Shaper (Oster Co., USA). Then the remaining hair was removed with a depilator, calcium thioglycolate ointment. The mice were divided into 9 groups. Five mice belonged to each group. In 4 groups, the back skin was exposed to 50mJ/cm² of UVB. The exposed sites of separate groups of mice were then injected intradermally with 10μCi of TdR-³H immediately after irradiation and at 6, 24 and 48 hours postirradiation.

In four other groups of mice, the back skin was exposed to 50J/cm² of UVA. The exposed skin was then injected with 10μCi of TdR-³H immediately after irradiation and at 6, 24, 48 hours postirradiation.

In a control group, 10μCi of TdR-³H was injected into the unirradiated back skin.

2) Autoradiography

One hour after injection of TdR-³H, the skin was biopsied and fixed in 10% neutral formalin. Tissue sections were coated with nuclear track emulsion type NTB-2 (Kodak, USA) under a safelight (Kodak Safelight Model B, Filter No 2) in a darkroom. Specimens were then exposed for 4 weeks at 4°C.

Subsequently the tissue sections were developed with Kodak developer D 19 (Kodak, USA) and fixed with Kodak fixer (Kodak, USA). They were then stained with H & E for light microscopy. Labeled cells were counted in a 5mm length of epidermis under high power (x400) light microscope with an eyepiece micrometer (Olympus Co., Japan). There were two kinds of labeled cells. One was HLC which had more than 15 grains. This was considered to be making DNA in S phase of the mitotic cycle. The other was SLC which had 3 to 15 grains. This represents UDS to repair the DNA damaged by UVL irradiation. The data obtained from the ex-

perimental and control areas were compared by statistical analysis using the Wilcoxon rank sum test.

RESULTS

UVA irradiation (Table 1)

1) heavily labeled cells (Fig. 1)

The number of HLC was slightly decreased, 61.00 ± 25.00 , immediately after UVA compared to the number in the unirradiated control, 93.40 ± 31.90 . Subsequently, 6 hours after UVA, the number was slightly increased to 108.20 ± 19.10 but this was not considered significant. The number was significantly increased to 131.00 ± 22.60 after 24 hours ($p < 0.05$). After 48 hours postirradiation the number recovered to 97.80 ± 39.20 .

2) Sparsely labeled cells (Fig. 2)

The number of SLC significantly increased to 34.40 ± 15.69 immediately after irradiation compared to the number of the unirradiated control, 13.30 ± 4.76 ($p < 0.05$). The significantly increased number was maintained 6 hours after

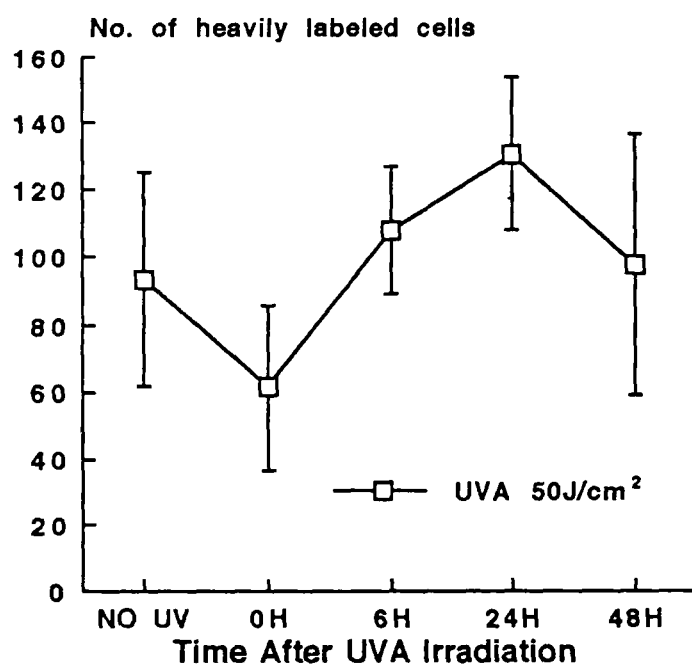


Fig. 1. Changes in the number of heavily labeled cells according to the time of UVA irradiation.

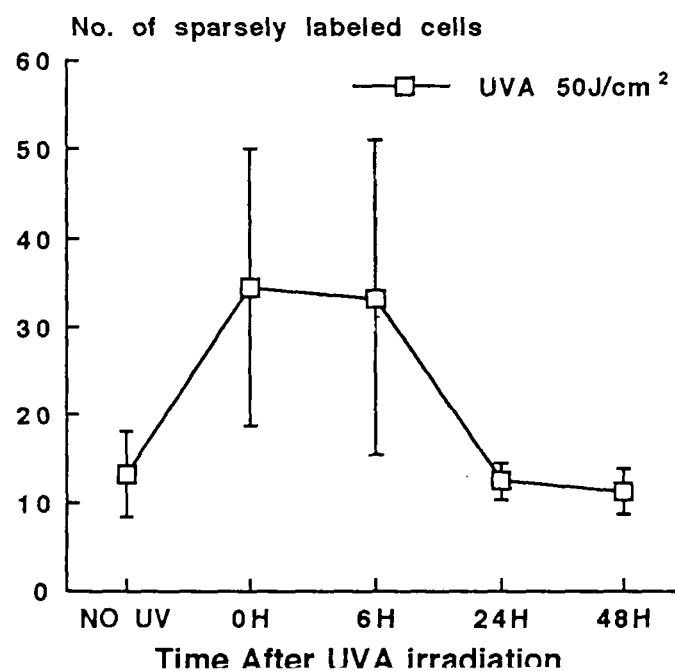


Fig. 2. Changes in the number of sparsely labeled cells according to the time of UVA irradiation.

Table 1. Changes in the number^a of labeled cells according to the time and type of UV irradiation

Time ^b and Type of UV	Heavily Labeled Cells (>15 grains)	Sparsely Labeled Cells (3-15 grains)
control	93.40 ± 31.90	13.30 ± 4.76
UVA 50J/cm ²		
immediate	61.00 ± 25.00	34.40 ± 15.69 ^c
6 hours	108.20 ± 19.10	33.20 ± 17.56 ^c
24 hours	131.00 ± 22.60 ^c	12.60 ± 2.07
48 hours	97.80 ± 39.20	11.20 ± 2.59
UVB 50mJ/cm ²		
immediate	26.80 ± 4.09 ^c	211.40 ± 48.50 ^c
6 hours	11.60 ± 8.53 ^c	63.60 ± 7.44 ^c
24 hours	30.60 ± 27.90 ^c	5.60 ± 2.51 ^c
48 hours	77.40 ± 44.30	10.80 ± 6.57

^a Mean ± SD

^b Time of intradermal ³H-thymidine injection after UV irradiation.

^c $p < 0.05$ relative to control (no UV irradiation) group.

UVA irradiation. Recovery was noticed 24 hours after irradiation and it was maintained after 48 hours postirradiation.

UVB irradiation (Table 1)

1) Heavily labeled cells (Fig. 3)

The number of HLC was significantly

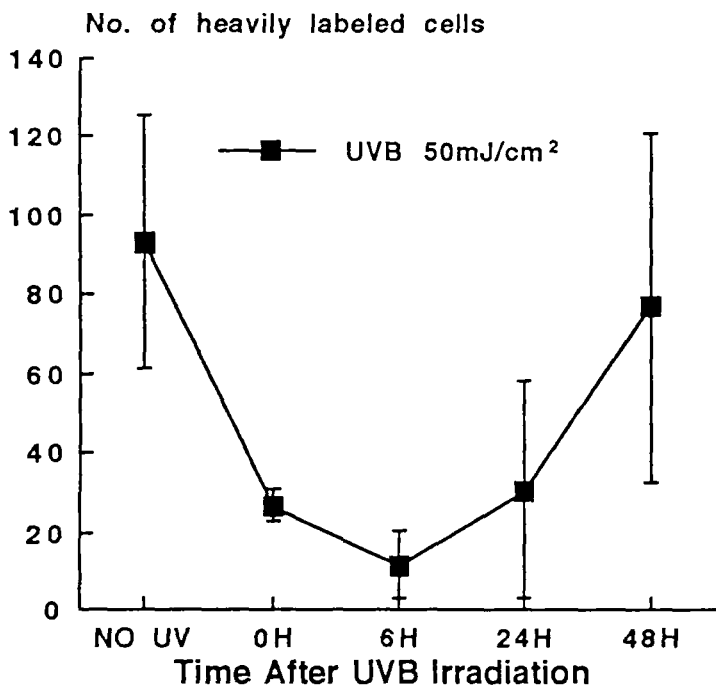


Fig. 3. Changes in the number of heavily labeled cells according to the time of UVB irradiation.

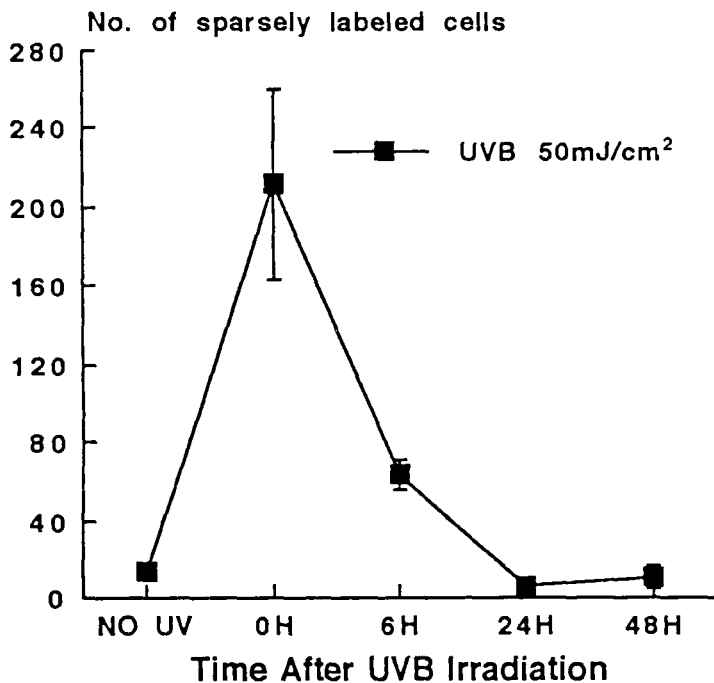


Fig. 4. Changes in the number of sparsely labeled cells according to the time of UVB irradiation.

decreased to 26.80 ± 4.09 immediately after UVB ($p < 0.05$) and was significantly decreased at 6 hours postirradiation to 11.60 ± 8.53 ($p < 0.05$). The significant decrease was maintained until 24 hours after UVB. The number recovered after 48 hours postirradiation.

2) Sparsely labeled cells (Fig. 4)

The number of SLC increased significantly to 211.40 ± 48.50 immediately after irradiation ($p < 0.05$). This was maintained 6 hours after irradiation ($p < 0.05$). The number of SLC decreased to 5.60 ± 2.51 after 24 hours postirradiation ($p < 0.05$). Complete recovery was noted after 48 hours postirradiation.

DISCUSSION

Light is essential for the survival of humans, animals and plants. The solar spectrum consists of UVL, visible light and infrared. UVL can be divided by spectrum into UVA, UVB and UVC. UVC does not reach the earth's surface. UVB is the most active wavelength for the production of erythema and pigmentation. It also causes a change in epidermal DNA synthesis and UDS which means excision repair of UVB induced DNA damage. The magnitude of change in DNA synthesis and UDS depends upon the wavelength, amount of UVB and the time after UVL (Hoenigsmann *et al.* 1981; Gange and Parrish 1983; Kochevar 1983).

In a previous study we studied the effect of $100\text{mJ}/\text{cm}^2$ UVB 24 hours postirradiation (Shin *et al.* 1990). A high pressure mercury arc was used as a source of UVB, but its spectrum of power distribution included UVA, UVC and visible range. We used a fluorescent sunlamp, which was recently used as a standard UVB source, as a UVB source in this experiment. (Harber and Bickers 1989). In this study HLC decreased immediately and at 6 hours after UVB exposure. The decreased number of HLC was maintained for 24 hours. The number recovered at 48 hours after UVB exposure. SLC increased immediately after UVB and 6 hours after. There was no increase in SLC after 24 hours and the number recovered after 48 hours. These results indicate that UVB caused depression in DNA synthesizing epidermal cells up to 24 hours postirradiation. They recovered 48 hours postirradiation.

UDS to repair pyrimidine dimers formed by injured DNA molecules occurred abruptly after UVB irradiation in large amounts. Increased

UDS persisted up to 6 hours after irradiation. Time course studies of UDS indicate that repair synthesis was completed 24 hours after UVB in this experiment but was still observed at 24 hours after UVB exposure using the high pressure mercury arc in our previous experiment (Shin *et al.* 1990). These differences may explain the wavelength dependency of UDS.

Early depression of DNA synthesizing cells was discovered in both experiments using different UVB sources. Early depression of DNA synthesizing cells persisted up to 24 hours in this experiment and recovered at 48 hours. However, depression recovered after 24 hours in our previous experiment (Shin *et al.* 1990) using a greater UVB dose ($100\text{mJ}/\text{cm}^2$) as it did in other reports using the high pressure mercury lamp (Epstein 1969). A shorter wavelength spectrum is produced by a high pressure mercury arc and therefore, depression of DNA synthesis by high pressure mercury arc recovered more quickly than that produced by the fluorescent sunlamp.

Although it had been generally accepted that UVA had little or no biologic effects, evidence to the contrary has already been presented (Willis *et al.* 1973; Kaidbey and Kligman 1978; Greaves and Briffa 1981). Controversial results have been reported on epidermal DNA synthesis and UDS by UVA. This was probably due to the fact that the dose, spectrum and source of UVA was not definite.

In this experiment we used a metal halide mercury lamp which produced strong UVA. From this UVA source we cut out UVB and UVC by using a UV filter. Most of the data on DNA synthesis and DNA repair in skin concerns after UVB, and only sparse data exists on the effect of UVA (Cripps *et al.* 1972; Hoenigsmann *et al.* 1981; Ambrosio *et al.* 1981; Kodama *et al.* 1984; Chew *et al.* 1988). In this experiment, we clearly demonstrated the effect of UVA on UDS. Immediately after and at 6 hours after irradiation of UVA, there was an increased number of SLC. $50\text{J}/\text{cm}^2$ UVA induced a considerable amount of UDS which suggested that DNA damage and repair had occurred in the mice. This damage

had already recovered 24 hours after UVA irradiation.

HLC decreased slightly immediately after UVA, but this was not significant. There was no decreased number of HLC at 6 hours, 24 hours and 48 hours after irradiation. These results demonstrate that there was no early depression of DNA synthesis immediately after $50\text{J}/\text{cm}^2$ UVA exposure and up to 48 hours postirradiation. Chew *et al.* reported the effect of various doses of UVA on DNA synthesis. There was no increased DNA synthesis after $40\text{J}/\text{cm}^2$ but decreased DNA synthesis was discovered after $60\text{J}/\text{cm}^2$ and $80\text{J}/\text{cm}^2$ UVA exposure (Chew *et al.* 1988).

UVB is about 1000 times more erythemogenic than UVA (Gange 1987). We used the same erythemogenic dose of UVA ($50\text{J}/\text{cm}^2$) compared to UVB in this experiment. The present data show that there are some differences in results between UVA and UVB. They are as follows. 1) Decreased DNA synthesis was noticed by UVB irradiation, but there was no decrease in exposure of $50\text{J}/\text{cm}^2$ UVA. 2) There was a clearly increased number of SLC by UVA irradiation. But the number of SLC was lower than the number by UVB irradiation immediately after and 6 hours after irradiation. This observation raises the question of the wavelength difference at the same erythemogenic dose between UVB and UVA.

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