Effects of UV Light on DNA Synthesis Studied by Autoradiography

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The status of DNA synthesis and the effects of UV radiation on DNA synthesis were studied in mouse skin by microautoradiography.

Mice exposed to UVB 100mJ/cm^2 were injected intradermally with tritiated thymidine 5 minutes after irradiation. We compared heavily labeled cells(HLC) and sparsely labeled cells(SLC) in UV irradiated skin with unirradiated control skin.

Before UV irradiation, the number of HLC, which are the DNA synthesizing cells, was 66.8±22.0 in 0.5cm of the basal layer. After UV irradiation the number of HLC was decreased to 27.4±9.8 in the basal cell layer. SLC were increased to 185.4±37.5 in the basal cell layer and to 109.8±19.9 in the differentiated cell layer.

This data suggests that unscheduled DNA synthesis was increased to repair the DNA damaged by UV radiation. (Ann Dermatol 1:6—9, 1989)

Key Words: Autoradiography, DNA synthesis, Unscheduled DNA synthesis, UV light

Ultraviolet light(UVL) exposure induces several biological changes. These biological changes include the manifestations of biologic responses of absorbed light by specific chromophores such as DNA.[1] Therefore, learning about the effect of UVL on DNA would be the basis for the study of the photobiologic response of UVL on the skin.

In recent years, the effects of DNA repair on cutaneous carcinogenesis and mutagenesis have received much attention. The survival of cells exposed to UVL is critically dependent upon their ability to repair damage of their DNA. The detection and quantitation of DNA repair by UVL is one of the major goals of photobiology.[2-5]

The pyrimidines are about 10 times more sensitive than purines to photochemical reaction by UVL.[6] Among various photochemical reactions of pyrimidines, thymine dimers have a unique chemical stability and can be isolated and assayed easily.[6] Therefore, UVL induced DNA repair could be detected by measurement of thymine dimers in skin. Futhermore, formation of thymine dimers could be assessed by measuring the rate of unscheduled DNA synthesis[7-9] or the number of thymine dimers.[2,10] Autoradiography is a method to assess unscheduled DNA synthesis, and this will provide information on the location of DNA damage.[7-9]

Autoradiography detects the localization of labeled radioactive materials in tissues such as skin. Quantitative assessment can be made by comparing the density of silver grains counted in skin specimen in microscopic autoradiography.[11,12]

Generally more than 85%, or 90% of the injected radioactive tracer, tritiated thymidine (TdR-^{3}H), in autoradiography is incorporated in the DNA molecule.[13,14] This was demonstrated by the distribution of TdR-^{3}H in chemically extracted DNA in human[13] and mouse skin.[14] This labeling pattern, which was incorporated in the DNA molecule, was eliminated by incubation with DNAase.

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Abbreviations:
HLC: Heavily labled cells
SLC: Sparsely labeled cells
HLB: Heavily labeled cells in basal layer
SLB: Sparsely labeled cells in basal layer
SLD: Sparsely labeled cells in differentiated layer

UVL irradiation of mammalian cells in tissue culture results in an incorporation of TdR-^{3}H into the nuclei of nearly all cells.[15]

Using microautoradiographic techniques, TdR-^{3}H labeling of epidermal cells can be counted in control tissues and ultraviolet irradiated tissues.

In autoradiography after UVL exposure, we can see two kinds of labeled cells.[13,14] The first one is heavily labeled cells(HLC), which are considered to be making DNA in the DNA synthesizing phase of the cell cycle. The second one is sparsely labeled cells(SLC) which represent incorporation of thymidine to replace the damaged DNA by UV irradiation. These 2'kinds of cells have already been observed in mouse skin[14] and human skin.[13] We studied the status of DNA synthesis and the effects of UVL on DNA systhesis in mouse skin.

MATERIALS AND METHODS

Materials

Experimental animals.

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

Light source

The UV radiation source was Burdick UV-800, a high pressure mercury arc (Burdick Co. USA). The UV radiation provided was mostly UVB range. An IL 700A research radiometer (International Light Co, USA) fitted with an Il broad-band UVB detector (SEE 240 International Light Co, USA) was used to measure the UV irradiance at skin level.

Radioactive tracer

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

Methods

The mice were divided into two groups. In one group of 5 mice, the back skin was exposed to 100 mJ/cm^2 of UVB. This dose of UVB was sufficient to develop sunburn cells in mouse skin in our previous experiment[16]. Within 5 minutes after UVL exposure, 10μ Ci of TdR-^{3}H were injected intradermally into the irradiated site.

In the control group, 10μ Ci of TdR-^{3}H was injected into the unirradiated back skin. One hour after injection, a skin biopsy was done and fixed in 10% formalin. Tissue sections were coated with nuclear track emulsion type NTB-2 (Kodak, USA 1988) under a safelight (Kodak safelight Model B, Filter No 2) in a darkroom. After exposure for 4 weeks at 4°C, tissue sections were developed with exposure to Kodak developer D19(Kodak, USA, 1988) for 5 minutes. Developed sections were fixed with Kodak fixer(Kodak, USA 1988). Tissue sections were stained with H&E for the light microscope. Labeled cells were counted in a 5mm length of epidermis before and after UV radiation under the high Power (×400—×1000) light microscope. We divided the position of the labeled cells into 2 sites, the basal cell layer and differentiated cell layer including the granular layer and malpighian layer.

RESULT

The HLC showed over 10 grains per nucleus(Fig.1), There were usually too many grains to count in HLC. The SLC showed 3-10 grains per nucleus.(Fig.2).

Before UV irradiation (Table 1, Fig. 3).

Before UV irradiation all of the labeled cells were HLC. The number of HLC was 66.8±22.0 in 0.5cm of the basal layer. The number of SLC was 0.9±0.7 in the basal layer (SLB), and 0.6±2.4 in the differentiated layer(SLD) (Fig. 4).

Table 1. Number of labeled cells before and after UV irradiation in 0.5cm epidermis

*Type of cells	Before UV	After UV
HLB	66.8±22.0	27.4±9.8
SLB	0.9± 0.7	185.4±37.5
SLD	0.6± 0.4	109.8±19.9

*HLB: Heavily labeled cells in basal layer
SLB: Sparsely labeled cells in basal layer
SLD: Sparsely labeled cells in differentiated layer

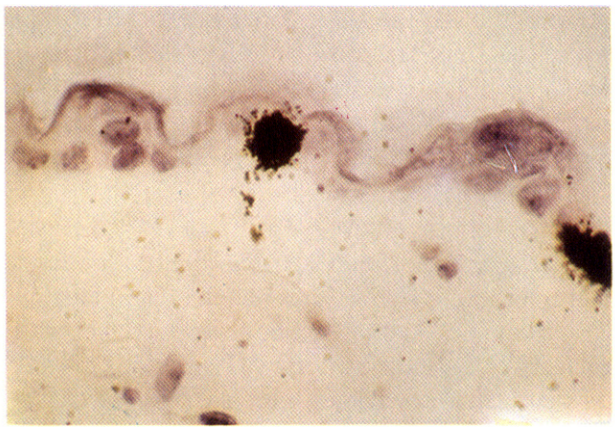

Fig. 1. Heavily labeled cells which show dense tritiated thymidine labeling of the nucleus (×100).

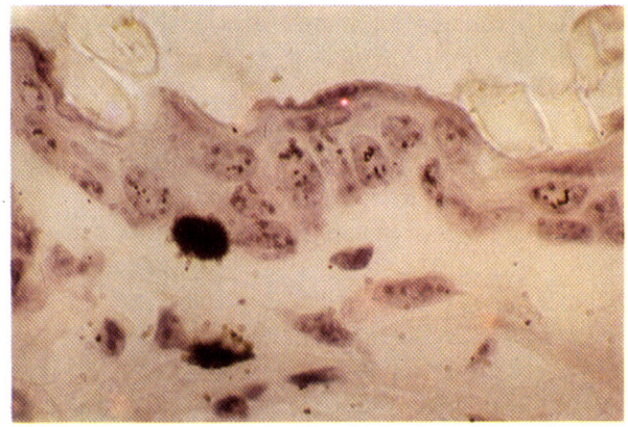

Fig. 2. Sparsely labeled cells which show 3-10 grains per nucleus (×100).

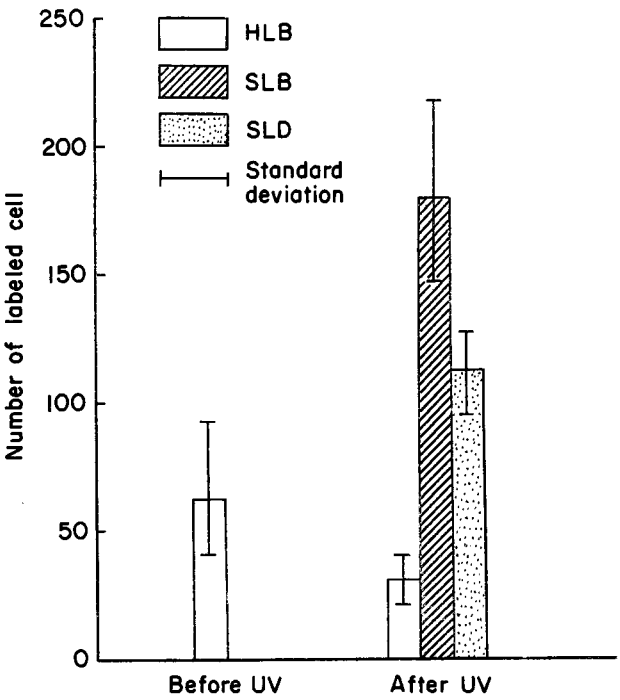

Fig. 3. Comparision of number of labeled cells before and after UV irradiation in 0.5cm epidermis.
*HLB: Heavily labeled cells in basal layer
SLB: Sparsely labeled cells in basal layer
SLD: Sparsely labeled cells in differentiated layer

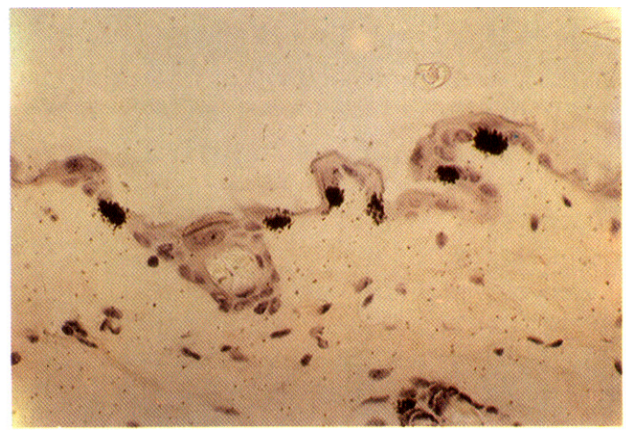

Fig. 4. Nonirradiated control skin of mouse showing dense labeling of basal cell nuclei in microautoradiography (×400).

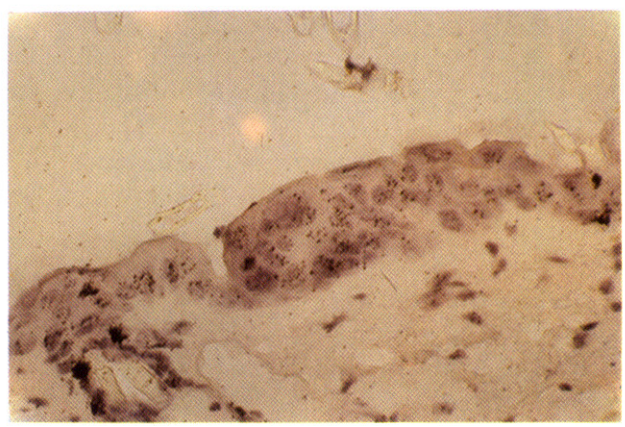

Fig. 5. Sparse labeling of nuclei in basal cells and differentiated cells is shown 5 minutes after UV irradiation (×400).

After UV irradiation (Table 1, Fig. 3).

The number of HLB was decreased to 27.4±9.8. There were no HLC in the differentiated cell layer. SLB were increased to 185.4±37.5 and SLD were 109.8±19.9(Fig.5).

DISCUSSION

Autoradiography is a method to detect the labeled radioactive tracer in tissues.[11,12] We used the TdR-^{3}H labeling method in this experiment to assess the status of DNA synthesis in normal and UV radiated mouse skin. Almost all of the HLC, which were considered to be in the DNA making phase of the mitotic cycle, were defined by the presence of more than 10 grains in the nucleus.[13,14,17] Almost all of the HLC were in the basal cell of the normal control skin. There were so many grains in almost all of the HLC that it was difficult to count the number of grains. In this experiment there were 66.8±22.0 labeled nuclei in 0.5cm of normal mouse skin. It is reported that approximately 5% of the basal cells in the hairless mouse epidermis synthesize DNA under normal conditions.[14] This data for normal conditions is a little higher than the report of Epstein et al.[14] They reported 53.83±16.92 labeled cells.

After UV radiation, the HLB were decreased to 27.4±9.8. This means that depression in DNA synthesis occurred after UV radiation. However, SLC were increased. The number of SLB was 185.4±37.5 and SLD was 109.8±19.9. This sparse incorporation of TdR-^{3}H in basal and differentiated cells indicates that UV light induced unscheduled DNA synthesis was increased. The unscheduled DNA synthesis represents excision repair of UV light damaged DNA molecules.[7-9] The thymine dimer formed by UV radiation is excised and thymidine is replaced into the existing DNA molecule[3-5] This repair replication has already been demonstrated in microorganisms and mammalian cells.[3,10,18] Cultured cells from patients with UV-sensitive xeroderma pigmentosum showed reduced levels of excision repair after UV radiation.[19] In this experiment we studied the short-term effects of UV light upon DNA synthesis. We think that a study of the long term effects of UV light would be necessary to observe the recover from the effects of UV light.

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