BIOLOGIC CHANGES DUE TO LONG-WAVE ULTRAVIOLET IRRADIATION ON HUMAN SKIN: ULTRASTRUCTURAL STUDY

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Alteration of the skin induced by single and repeated long-wave ultraviolet (UVA) exposures was studied. Following a single exposure to relatively large doses of UVA, pronounced dermal damage was observed. In the papillary dermis, superficial dermal vessels showed widely open endothelial gaps and extravasation of blood cells. Marked changes of fibroblasts were also seen in the superficial dermis. In the reticular dermis, extravascular fibrin deposition was seen. After repeated exposures to UVA the formation of cross-banded filamentous aggregations (“Zebra bodies”) was observed in the superficial and reticular dermis. These were often found in amorphous masses surrounding the blood vessels. These striking dermal alterations were absent in skin irradiated by solar stimulating radiation and in control skin. Dyskeratotic “sunburn cells” were occasionally seen in the epidermis after single as well as repeated exposures to UVA. The number of these cells was less than that seen after a single exposure to solar stimulating radiation.

A single exposure of the normal skin to long-wavelength ultraviolet (UVA) has generally been believed not to cause visible erythema, unless a relatively high dose (20–90 joules/cm²) is given [1–4]. Histologic examination using the light microscope does not reveal any significant change in the irradiated skin. However, with more precise methods subtle biologic effects of UVA have been detected: Willis, Kligman, and Epstein [5] noted a transient acceleration of DNA synthesis within 24 hr and a mild stimulation of unscheduled DNA synthesis. In abnormal conditions such as various types of photodermatoses, a nonhematine type of inflammation was elicited by UVA (4–14 J/cm²) [6,7]. A persistent light reactor was reported to respond to UVA (3–4 J/cm²) with a mild perivascular infiltration of lymphocytes and the formation of “sunburn cells” [8]. These findings suggest that UVA alone as well as combined with other factors may induce some alterations in the skin, even if it requires from 10 to 100 times the dose of middle-wavelength ultraviolet (UVB) to induce detectable changes in the normal skin.

Although UVA usually does not cause erythema [9–11] and has no effect on isolated cutaneous blood vessels [12], it is of greater intensity on the earth than UVB [13]. It penetrates the dermis deeper and in much greater quantity than does UVB [13–15].

UVA combined with 8-methoxypsoralen has been used recently in the treatment of psoriasis (PUVA therapy) and Konrad et al [16] reported sunburn reactions in the skin following PUVA. However, the histologic effect on the skin of UVA at the ultrastructural level is entirely unknown. The present study was undertaken to elucidate at an ultrastructural level injuries caused by single and repeated UVA irradiation, and the results were compared with the effect of solar simulating radiation (SSR).

MATERIALS AND METHODS

Subjects. These were 5 white, adult male volunteers. The midportions of their backs served as the test sites. A total of 34 biopsies were obtained from them. The specimens were taken with 2-mm skin punches and cut into 1-mm² pieces.

Light sources. SSR in the range of 290 to 420 nm was obtained from a xenon lamp after the design of Berger [17]. UVB was eliminated by a 2-mm Schott WG345 filter, which has a transmission of 50% of its peak at 345 nm and cuts off practically all rays below 320 nm (i.e., <0.01% transmission at 315–320 nm). We hereafter refer to this as UVA radiation although a very small amount of near-visible energy is also present (<0.022% at 400–420 nm). The latter can evoke immediate pigment darkening (IPD) as Pathak and Stratton have shown [18].

Energy measurements were obtained with an International Light spectroradiometric system calibrated at 1 meter by a 1,000-watt quartz halogen lamp traceable
to the U.S. National Bureau of Standards. Total energies emitted at the point where the subjects were irradiated were $1.8 \times 10^5 \mu$W/cm$^2$ of UVA (through the Schott WG345 filter) and $2.5 \times 10^6 \mu$W/cm$^2$ of SSR.

For the light source of a monochromatic light, a 1600-watt xenon arc lamp was used. The light was filtered through a high-intensity grating monochromator (Bausch and Lomb at 350 nm (10-nm band pass) and then filtered through an 0.005D Mylar filter. There was no heat generated during the exposure periods, and there was no stray UV present in the light that reached the subject’s skin.

**Single exposures.** Two volunteers (#1 and #3) were irradiated with UVA and SSR in different areas (each area measuring 1 cm$^2$) by solar simulator. Volunteer #1 was irradiated with UVA (0, 86, and 173 J/cm$^2$) and with SSR (23 J/cm$^2$). Forty-eight hours after exposure, biopsies were taken and fixed in 5% glutaraldehyde in 1/10 m cacodylate buffer adjusted to pH 7.4. Fixation took 4 hr. Volunteer #3 was irradiated with UVA (0 and 130 J/cm$^2$) and with SSR (15 J/cm$^2$) by the solar simulator. Biopsies were taken after 15 min and 24 hr from each area and fixed in the same manner using glutaraldehyde. Volunteers #4 and #5 were irradiated with monochromatic light of 350-nm band (0 and 42.8 J/cm$^2$). Biopsies were taken at 72 hr postirradiation and fixed in the same glutaraldehyde.

**Repeated exposures.** UVA and SSR obtained by solar simulator were administered to each site approximately every 2 weeks for a period of almost 1 year. During this period, volunteer #2 was exposed 17 times, receiving each time 112 J/cm$^2$ of UVA and 13 J/cm$^2$ of SSR. Thus, the total dose received by volunteer #2 was $1.9 \times 10^5$ J/cm$^2$ of UVA and $2.2 \times 10^6$ J/cm$^2$ of SSR.

Volunteer #3 was exposed 20 times, receiving each time 130 J/cm$^2$ of UVA and 15 J/cm$^2$ of SSR. Totally, he received $2.6 \times 10^5$ J/cm$^2$ of UVA and $3 \times 10^6$ J/cm$^2$ of SSR. The irradiated areas of volunteer #2 were biopsied 5 days after the last exposure and those of volunteer #3 were biopsied 15 days after. All specimens were immediately fixed in the same glutaraldehyde. Nonirradiated control specimens were also biopsied and similarly fixed.

**Electron microscopy.** The tissues fixed in glutaraldehyde were postfixed in 1% osmium tetroxide in 0.1 m cacodylate buffer (pH 7.4). Routine methods were used for embedding, thin sectioning, and staining [19]. Thin sections were observed in an Hitachi HU-11C electron microscope at an accelerating voltage of 100kv.

**RESULTS**

After a single exposure to UVA, IPD was observed on each site irradiated by both solar simulator and monochromator. Marked erythema was observed immediately after exposure in all sites irradiated by solar simulator and was present at 48 hr when biopsy specimens were obtained. In the experiments conducted with monochromatic light, 1 minimum erythemal dose (MED) responses were apparent at 24 hr postirradiation with 42.8 joules.

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- A summary of 24 biopsies from 4 volunteers.
- A summary of 10 biopsies from 2 volunteers.
- Solar simulator with filter is used as a light source.
- Monochromator is used as a light source. +++ = Strongly positive; ++ = moderately positive; + = weakly positive; = negative.
of 345-355 nm light. On a single exposure site of SSR, biphasic erythema was observed. After a repeated exposure to UVA and SSR by solar simulator, the skin appeared to be normal except that the color changed to brown. No abnormality was seen in the control skin.

**Electron Microscopic Observations (Table)**

A single exposure of UVA. A high dose of exposure (173 J/cm²) by solar simulator showed quantitatively more severe change than 86 J/cm² (1 MED of UVA [4]) exposure, but qualitatively the same alteration was seen. Also, the skin irradiated by the 350-nm band of the monochromator (42.8 J/cm²) showed the identical changes. In both experiments, changes of keratinocytes were minimal, consisting of lipid droplet formation, focal widening of intercellular spaces, and vacuole formation. Occasional "sunburn cells" were dyskeratotic keratinocytes which had aggregated tonofibrils around the pyknotic nuclei. However, the majority of the keratinocytes appeared unaltered. Melano-

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**Fig. 1.** Platelets (*) are aggregated in the lumen. Widely opened endothelial gaps (thick arrow) and extravasation of a red blood cell (R) are seen. Biopsy was taken 48 hr after a single exposure to UVA by SSR (volunteer #1) (× 8,700). Scale = 2,000 nm.
cytes and Langerhans cells were intact, except that sometimes one or two lipid droplets and widening of intercellular spaces were seen.

The most characteristic changes were observed in the blood vessels in the papillary and reticular dermis. These changes were opening of endothelial junctions, platelet aggregation, and extravasation of blood cells (Figs. 1–4). In the superficially located vessels, these changes were observed in almost all vessels, whereas in the deeper dermis the changes were irregular and generally milder. In the lumen there were platelet aggregates (Fig. 1) and a threadlike substance which appeared similar to fibrin crystallization (Fig. 2). Sometimes, densely packed red blood cells were seen in small vessels. Frequently, the opening of an endothelial junction was so pronounced that the gaps between endothelial cells became wide and basal lamina was directly exposed to the capillary lumen (Figs. 2, 3). Through these widely opened gaps, blood cells apparently escaped (Figs. 1, 2). Pericytes often showed cytoplasmic changes and pyknotic nuclei (Figs. 2, 4). These changes were also observed in fibroblasts which were in the papillary dermis.

Fig. 2. Gap (*) between endothelial cells becomes wide and basal lamina is directly exposed to the capillary lumen. Pericyte (P) has a pyknotic nucleus. R = Red blood cell. Biopsy was taken 48 hr after a single exposure to UVA by SSR (volunteer #1) (× 7,200). Scale = 2,000 nm. Inset: Enlargement of gap (*) between endothelial cells. Threadlike substances (arrow) are seen in the lumen (× 18,000). Scale = 500 nm.
Leukocytic granules were present around the affected vessels (Fig. 4). Leukocytes which had condensed cytoplasm and pyknotic nuclei were sometimes seen in the reticular dermis, and were occasionally phagoyctized. Fibrin deposition was distributed among the collagen bundles was found in the reticular dermis (Fig. 5), but not in the papillary dermis. Fibrin fiber had peculiar cross-banding (Fig. 5, inset). Mild to moderate degranulation of mast cells was seen.

A single exposure of SSR. After SSR irradiation, there were epidermal and dermal alterations. In contrast to the findings in the tissue obtained after a single UVA irradiation, the changes were more severe in the epidermis and less so in the dermis. Dyskeratotic cells and other cytoplasmic changes were almost the same as those caused by UVB irradiation [20–24] and dermal changes were milder. Dilatation of small venules and capillaries was found. Mast cells were seen near these dilated venules, but no prominent degranulation was observed. Occasionally, a few lipid droplets were present in the cytoplasm of a fibroblast, but there were no other alterations.

Repeated (17–20 times) exposure of UVA. The epidermal changes were a little more prominent than those of a single exposure. Vacuoles were formed in the lower epidermis, and lipid droplets were seen. Dyskeratotic cells, or sunburn cells, were occasionally seen in the basal layer. A great number of melanosome complexes were present in the basal keratinocytes and sometimes distributed in the form of a characteristic nuclear cap. Basal lamina of the epidermis was occasionally multilayered.

Blood vessels and lymphatics of the upper dermis showed characteristic changes (Figs. 6, 7). In the subendothelial area and sometimes among the collagen bundles in the perivascular spaces, there was a heavy deposition of amorphous, medium electron-dense substance in which the width of the basal lamina was about 100 nm, almost the same as the control. Within this amorphous material, the formation of cross-banded fibrous aggregations, Zebra bodies, was seen. These aggregations had a periodical banding with 100-nm intervals as compared with the periodicity of 64 nm of native collagen (Fig. 8). Fibroblasts had an enlargement of endoplasmic reticulum and produced half-desmosome-like structures on their cytomembranes.

Repeated (17–20 times) exposures of SSR. In the epidermis, only a few changes were observed, i.e., lipid droplet formation and focal widening of intercellular spaces. Basal cells had many melanosome complexes. No dyskeratotic cells were seen. Compared to the skin that has received a single exposure to SSR, there was marked thickening of epidermis. The deposition of amorphous materials was also seen around the small vessels on the upper dermis, although not to the amount seen in UVA-irradiated skin. Zebra bodies were rarely found.

Control tissues. No abnormalities were detected except occasional fixation artifacts such as very mild vacuolar changes in keratinocytes and focal widening of intercellular spaces. These were within the expected frequency. Blood vessels were entirely normal and the structures of endothelial cells, pericytes, fibroblasts, and mast cells were intact.

**DISCUSSION**

It has been known that UVB induces sunburn effects, and a variety of degenerative changes have been reported in epidermal keratinocytes; these include intracytoplasmic edema, vacuole formation, lipid droplet formation, and individual cell keratinization, namely sunburn cells [20–24]. Elevated permeability of blood vessels due to vascular injury was observed in the superficial capillary network, as reported by several investigators [25–27]. Specimens irradiated by SSR were reported to demonstrate the same epidermal changes as those found in UVB-treated skin by light microscopy [5].

On the other hand, the effects of UVA were reported to cause no or minimal changes [5,27,28]. However, in our present studies using high UVA doses, it was found that the epidermis responded with only mild changes after a single exposure to 1 to 2 MEDs of UVA [4], and much more pronounced endothelial cell damage was produced by UVA. Otherwise, full-spectrum UV (i.e., UVA + UVB or SSR) induced only mild vascular changes even with an equally erythemogenic dose [5], but caused severe epidermal damages. UVA is less absorbed in the epidermis and penetrates deeper in the skin than UVB [13–15] so that the ultrastructural changes found in the present study were compatible with these properties.

Injuries in blood vessels were classified by Co-
Fig. 4. There are leukocytic granules (g) and destructive changes of a pericyte (P) in the superficial capillary. Asterisk = Platelet. Biopsy was taken 48 hr after a single exposure to UVA by SSR (volunteer #1) (× 7,500). Scale = 2,000 nm.

Fig. 5. In the reticular dermis, there is a heavy deposition of fibrin (f) among the collagen bundles. Biopsy was taken 48 hr after a single exposure to UVA by SSR (volunteer #1) (× 6,500). Scale = 2,000 nm. Inset: Enlargement of fibrin shown with asterisk in main figure. Peculiar cross-banding is seen (× 35,000). Scale = 100 nm.

tran and Majno in 1964 [29]. They described three types: (1) histamine-type leakage, (2) direct vascular injury, and (3) delayed prolonged leakage. The UVA-induced vascular damage seems to be classified with the second type. The "direct vascular injury" may be caused by any bacterial, physical, or chemical agents and can damage the vascular wall directly. Ultrastructure of the direct vascular injury consists of striking changes in the walls of the superficial vessels (arterioles, venules, and capillaries), and occluding platelet thrombi in severe cases. These changes were found in UVA-irradiated skin specimens as described in the present study. Sunburn caused by UVB is mediated by prostaglandin [30] and is decreased or delayed by antiprostaglandin agents [31], and this type of vascular injury has been shown to be associated with delayed prolonged leakage [29].

Since direct vascular injury was also caused by heat, the light source and intensity were designed so that the solar simulator was used to obtain UVA. In order to avoid heat effects, the intensity of $1.8 \times 10^6 \mu W/cm^2$ (which contained less than $1.8 \times 10 \mu W/cm^2$ of light energy below 320 nm) and 8 and 16 min duration (below the level of average heat erythema threshold [17]) was used. As shown in Results, however, marked erythema was observed immediately after exposure and continued at least 48 hr. Although this erythema was believed to be heat erythema, a preliminary study
showed that the same subjects' skin, exposed to UVA of only slightly smaller amounts than used in this study, resulted in UVA heat erythemas which vanished within 1 hr with no delayed vascular response. Therefore, there was an overlap of UV heat and true delayed UV erythema in this study. Irradiation with 350 nm region (a 10-nm band pass) obtained by monochromator was also used as UVA without resultant heat erythema. We consider, therefore, that the major effects were caused by UVA and not by heat.

Biopsies following multiple exposures were done 5 to 15 days after irradiation to avoid the acute changes. In these specimens a heavy deposition of amorphous masses around the vessels was the most characteristic feature. The thickness and the number of layers of basal lamina in these masses seemed to be within the normal range [32,33]. However, amorphous masses were extremely thick around the blood vessels and lymph vessels. Although the origin of basal lamina is not well established, the endothelial cell seems the most likely source [33,34]. Therefore, this functioning of endothelial cells was assumed to be not greatly disturbed in spite of a recurrent exposure to UVA. Lipid droplets or cellular debris were rarely seen. They were observed in chronic UVA-irradiated skin of erythropoietic protoporphyria (EPP) in which the main target was believed to be endothelial cells [27,28,35,36].

The cross-banded filamentous aggregations or so-called Zebra bodies were often seen in these amorphous masses. These aggregations have previously been found in normal and abnormal tissue of the central nervous system, but they were also found in the stroma of skin tumors, in various skin diseases [37,38], and were synthesized in a tissue culture system of fibroblasts [39]. It is probable that these aggregations and amorphous masses could be produced by fibroblasts (which showed active fibrillogenesis surrounding the blood vessels) to protect the blood vessels from the repeated exposure to UVA.

REFERENCES

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