DIFFERENTIATION OF HISTOLOGICAL CHANGES INDUCED BY ULTRAVIOLET-A AND -B LIGHT IRRADIATION IN THE AURICULAR SKIN AND EYE OF ALBINO BALB/C MICE

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Abstract: We differentiated the histological changes induced by irradiation with ultraviolet-A (UVA) and -B (UVB) light in the auricular skin and eye in female Balb/c mice. The animals were irradiated (1.30 mW/cm²) with UVA for 1, 3, or 7 days, or with UVB for 2, 4, or 8 hr or 1, 3, or 7 days. Irradiation with UVA and UVB induced inflammation in the auricular skin, but the early changes were different. UVA-irradiation caused neutrophil infiltration without apparent edema, while UVB-irradiation induced marked edema with degranulation of mast cells and enlargement of endothelial cells of blood vessels in the dermis. These lesions became more severe with time progression, with the auricle showing partial necrosis at the later stage. UVA-irradiation caused retinal degeneration with vesiculation of the photoreceptor outer segment as an early change. In contrast, UVB-irradiation initially induced degeneration of corneal cells, followed by degeneration of lens epithelial cells. Although the incidence of retinal changes induced by UVB-irradiation was much lower than that by UVA, the number of migrating cells in the photoreceptor segments with UVB significantly increased at the later stage. (J Toxicol Pathol 8: 407–415, 1995)

Key words: Phototoxicity, Ultraviolet-A, Ultraviolet-B, Auricular skin, Retina

Introduction

Recently, photodamage due to solar light, especially ultraviolet (UV) light, has become the focus of special interest because of ozone depletion. UV light has a wavelength ranging from 200 to 400 nm and is classified into 3 types, namely ultraviolet-A (UVA, 320 to 400 nm), -B (UVB, 290 to 320 nm) and -C (UVC, 200 to 290 nm). UVC is prevented from reaching the earth by the ozone layer, but UVA and UVB do reach the earth. While the amount of UVA reaching the earth has been described as 10 to 100 times greater than that of UVB, its potential to induce skin erythema in humans is thought to be 1,000 times less than that of UVB. Each photon of short wavelength-UV light has enough energy to excite an electron and induce photochemical reactions, which damage the constituents of protein and nucleic acid and result in lesions in the skin and eyes. Overexposure to solar light, particularly UVB, induces erythema, edema, suntan, sunburn dermatitis, and cancer in the skin and corneal damage and lens cataracts in the eye. Animals are also reported to show retinal degeneration on exposure to UV light; many reports have described UV light induced changes in the photoreceptor and retinal pigment epithelial (RPE) cells in monkeys, rats, and mice. Because mice lack yellow pigments in the lens, they are more sensitive to UV light than humans and easily develop retinal lesions. Moreover, albino mice and rats are susceptible even to standard illumination used in animal rooms. These animals sustain...
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Retinal degeneration and/or cortical cataract\(^{16-19}\), with such damage reported at an incidence of 30% in Balb/c mice\(^{20}\).

Each structure of the eye is characterized by its absorption of light of a specific wavelength. The cornea is reported to absorb wavelengths from 200 to 295 nm\(^{21-23}\) and to be most sensitive to those from 260 to 280 nm\(^{2}\), because the radiant energy is completely absorbed in this range\(^{24,25}\). Photokeratitis has also been reported to be induced by UV light in this range\(^{15,26}\). The lens contains chromophores which absorb UV ranging from 295 to 400 nm\(^{3,21}\). In particular, light with a narrow wavelength from 290 to 325 nm causes acute cataract in primates\(^{3}\). Although only less than 1% of UV light is thought to reach the retina\(^{27}\), light of 320 nm or longer has been reported to induce retinal degeneration\(^{8,19}\). Therefore, UVA generally induces changes in the retina, but not the cornea\(^{24,25}\).

The primary target in a tissue is thought to be determined by a combination of exposure parameters of UV light including wavelength, peak power, pulse width, pulse repetition rate, and total energy\(^{2}\). Although many reports have described photodamage in the eye and skin of animals, the characteristics of UVA- and UVB-induced histological changes are difficult to determine from published reports because the exposure parameters and animal species used were different. Additionally, few papers have reported the effects of different kinds of UV light at the same time. In the present study, we differentiated the histological changes induced by UVA and UVB light in the auricular skin and eye albino Balb/c mice.

Materials and Methods

Animals

Seventy-five female Balb/Crj (Balb/c) mice aged 8 weeks were purchased from Charles River Japan Inc. (Atsugi). They were housed in wire mesh cages in an air-conditioned room (temperature, 23±2°C; relative humidity, 55±15%; light cycle, 12 hr/day) for several days before use to acclimate them to the environment. The illumination intensity measured in cages was about 30 lux, and no UV light was detected (<0.01 mW/cm\(^2\)). Commercial laboratory rodent chow (F-2, Funabashi Farm Ltd., Japan) and tap water were available ad libitum.

Light sources

Black light tubes (FL20SBLB) and sun lamp (FL20SE) were purchased from Toshiba Co., Ltd. (Japan). These radiated wavelengths from 300 to 400 nm (peak at 360 nm) and 250 to 400 nm (310 nm) and were used as the sources of UVA and UVB, respectively. The intensities of UVA and UVB were measured by a UVX Digital Radiometer (UVP Inc., USA) and expressed as mW/cm\(^2\) at 365 and 315 nm, respectively.

Experiments

Animals were divided into 3 major groups, a UVA-(15 animals/3 subgroups) and UVB-(30/6) irradiation group and a control (30/6) group. The animals were placed individually in partitioned chambers (4×8×4 cm). The UVA group were covered with a 3-mm pane of glass to eliminate wavelengths below 320 nm, and the UVB group with a 3-mm quartz glass, which did not eliminate these wavelengths. The UVA and UVB lights were irradiated at the same intensity of 1.30 mW/cm\(^2\), with less than 0.1 mW/cm\(^2\) of UVB and UVA light passing through the glasses, respectively. The animals were adapted to the dark for 12 15 hr before the commencement of irradiation. Laboratory chow and tap water were available in the chamber during the irradiation.

Six groups were irradiated with UVA or UVB light for 12 hr/day (7:00–19:00 or 8:00–20:00) for 1, 3 or 7 days, and a further three groups with UVB for 2, 4 or 8 hr to explore the development of lesions of the auricular skin and retina. Animals irradiated for 1, 3 or 7 days were killed on the day after the final day of irradiation and those for 2, 4 or 8 hr immediately after irradiation by bleeding under ether anaesthesia. The control groups, maintained under the standard lighting in the animal room, were killed simultaneously with the respective irradiation groups. Total doses of UVA and UVB were 56 (1 day), 168 (3 days), and 393 (7 days) joules/cm\(^2\), and those of UVB were 9 (2 hr), 19 (4 hr) and 37 (8 hr) joules/cm\(^2\). On the
day before commencement of irradiation, auricular thickness was measured once a day during the irradiation period and immediately before killing using a dial thickness micrometer gauge (Peacock G-5, Ozaki MFG, Japan).

Histological examination

The auricles and eyes were removed immediately after killing, fixed in 10% buffered formalin and Davidson’s solution, respectively, dehydrated in graded ethanol, embedded in paraffin wax, sectioned, stained with hematoxylin and eosin and examined histologically. Toluidin blue stain was applied to a separate section of the auricle to observe mast cells. Moreover, the number of migrating cells in the photoreceptor outer segment (POS) of the retina was counted, and the length of Bruch’s membrane was measured on the slide using an image analyzer (LUZEX 5,000 X, Nireco Co., Ltd., Japan) and a microcomputer (PC-98 XL model, NEC Co., Ltd., Japan). The number of migrating cells per the length of membrane (×mm⁻¹) was calculated for each eye.

Statistical analysis

Differences in migrating cell numbers and auricular thickness between the control and irradiation groups were statistically analyzed using Duncan’s multiple range test.

Results

Auricles

Time course of the changes in auricle thickness induced by UVA and UVB irradiation is shown in Fig. 1. UVA irradiation increased auricular thickness from day 3. Thickness continued to gradually increase thereafter and became about 3 times greater than that before commencement of irradiation on day 7. Corresponding to the auricular thickening, erythema was seen from day 3 and a partial defect of the auricle tip on day 7. In contrast, UVB irradiation induced auricular thickening in 2 hr. This thickening reached a peak on day 2 and then gradually declined. The auricle exhibited erythema within 2 hr, a whitish discoloration on day 3 and hardening with partial loss of the tip on day 5, which made the measurement of thickness impossible thereafter. Furthermore, the animals developed a golden discoloration of the haircoat and erosion of the tail skin, and 1 animal died on day 7.

Histological findings are described below.

UVA: The epidermis contained many degenerated cells with pyknotic or clear nuclear matrix on day 2. A few animals showed foci of mild neutrophil infiltration in the dermis without apparent edema. On day 4, the number of the
degenerated cells with slightly decreased cytoplasmic staining was markedly increased in the epidermis. The dermal changes were also enhanced and consisted of moderate neutrophil infiltration and mild edema (Fig. 2). On day 8, necrosis of the epidermal cells was seen in small areas near the auricle tip and the horny layer was detached from the surface. The epidermis was thickened in the middle and root portions of the auricle, and the cells generally showed larger nuclei with several prominent nucleoli and were separated from each other by narrow spaces, particularly in the basal layer. The dermal tissue adjacent to the necrotic epidermis became necrotized in small areas, which were homogeneously eosinophilic and contained many nuclear fragments. In other areas of the dermis, histiocyte-like cells, fibroblasts and neutrophils were markedly increased in number, and a small number of eosinophils were also seen, while the edema became focal (Fig. 3).

UVB: A 2-hr irradiation-produced degeneration of epidermal cells, particularly at the auricle tip. There was severe edema with mild neutrophil infiltration and a remarkable degranulation of mast cells in the dermis. Mast cells were occasionally degenerative and surrounded by neutrophils (Fig. 4). Blood vessels showed enlarged endothelial cells and dilation and contained fibrillar substances and neutrophils in the lumen. At 4 hr, the changes became more severe. At 8 hr, epidermal cells considered to have been directly irradiated showed a marked decrease in cytoplasmic staining and were desquamated. On day 2, the subepidermal area was degenerated, with necrotic cells in the dermis of 1 case. In the dermis, disruption of necrotic walls of congested blood vessels, thrombi, and hemorrhage were seen in addition to severe edema and neutrophil infiltration. On day 4 and later, necrosis became more prominent and extensive in both the epidermis and dermis. On day 8, the auricles became shrunken.

Eyes

The number of migrating cells in the POS are shown in Table 1. Mean values on day 2 were 0.33 to 0.41 × mm⁻¹ in all experimental groups, including the control. With UVA and UVB irradiation the values increased gradually and significantly as the irradiation progressed, reaching 3.52 and 2.97 × mm⁻¹ on day 8, respectively. However, there were clear differences in the quality and severity of histological changes of the retina as described below.

UVA: Vesiculation or an edematous appear-
Table 1. Number of Migrating Cells in the Photoreceptor Outer Segment Per Length of Bruch's Membrane (×mm⁻¹) in the Retina of Mice Irradiated with Ultraviolet-A (UVA) or Ultraviolet-B (UVB) Light for 1, 3, or 7 Days

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>UVA</th>
<th>UVB</th>
</tr>
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<tbody>
<tr>
<td>1 day</td>
<td>0.41±0.41</td>
<td>0.41±0.22</td>
<td>0.34±0.28</td>
</tr>
<tr>
<td>3 days</td>
<td>0.22±0.21</td>
<td>0.91±0.62**</td>
<td>1.28±0.56**</td>
</tr>
<tr>
<td>7 days</td>
<td>0.24±0.25</td>
<td>3.52±1.60**</td>
<td>2.97±1.18**</td>
</tr>
</tbody>
</table>

*, period of irradiation.

b, values are presented as mean±SD of 5 animals per group.

**, significantly different (P<0.01) from the control group.

ance was seen mainly in the fundal POS of the retina in 2/10 eyes on day 2. These changes were associated with swollen RPE cells in the affected areas. Changes on day 4 were similar to those on day 2, with the PRE cells showing the most remarkable changes. The vacuolated PRE cells were slightly swollen and occasionally contained eosinophilic or brownish substances in the cytoplasm. Seven-day irradiation induced severe changes in 9/10 eyes. The changes on day 4 showed progression and the retina showed so-called arcade formation, that is, partial detachment of the retina from the RPE cell layer with a wavy arrangement of the outer and inner nuclear layers (ONL and INL) (Fig. 5). Cells migrating into the POS were swollen.

UVB: UVB irradiation produced marked changes in the other structures rather than in the retina. At 2 hr, corneal epithelial cells showed irregularly shaped nuclei, particularly in the central area. Additionally, weakly eosinophilic substances were seen in the anterior chamber. The corneal changes were exacerbated at 4 hr. The epithelial cells were dissociated from each other with a few necrotic cells (Fig. 6), and the squamous cells were partially desquamated. The stromal layer was slightly basophilic and thickened, being associated with neutrophil infiltration in the marginal area. At 8 hr, the corneal epithelial cells were necrotized in all layers, and some eyes showed many vacuoles distributed close to the Bowman's membrane. The corneal stromal and endothelial cells lost their nuclei. Moreover, some animals showed necrosis of the iris tip and pyknosis or disappearance of nuclei in the lens epithelial cells at the front areas. On day 2, the corneal epithelial cells were proliferated and more multilayered than

Fig. 5. Retina of Balb/c mouse irradiated with ultraviolet-A for 7 days. Retina shows partial detachment (*) from the pigment epithelial (RPE) cell layer with outer (ONL) and inner nuclear layers (INL) (arcade formation). HE stain. Bar=50μm.

Fig. 6. Cornea of Balb/c mouse irradiated with ultraviolet-B for 4 hr. The epithelial cells are dissociated from each other and slightly eosinophilic substances are seen in the anterior chamber. HE stain. Bar=50μm.
the control eyes. In addition to neutrophil infiltration, vascular penetration into the stroma was seen at the marginal areas of the cornea. Many lens epithelial cells had fragmented nuclei and small cytoplasmic vacuoles at the bow areas. On day 8, fibrillar substances and a small number of neutrophils were observed in the anterior chamber and vitreous, and the anterior chamber was somewhat narrowed. The necrosis of the iris and lens epithelial cells also became more prominent, and vacuoles sometimes developed at the bow areas in the lens (Fig. 7). In contrast, retinal changes were scarce and mild, with vesiculation of the POS observed in only 1 eye each on days 2, 4, and 8.

Discussion

UVA- and UVB-induced inflammation in the mouse auricular skin was differentiated in the present study. Early change induced by UVA irradiation was characterized by mild neutrophil infiltration without apparent edema in the dermis. As the irradiation progressed, the neutrophil infiltration became more pronounced with severe edema and thickening of the auricle. The later stage was characterized by high cellularity consisting of histocyte-like cells, fibroblasts, and neutrophils with reduced edema. In contrast, early changes with UVB consisted of marked edema with mild infiltration of neutrophils, degranulation of mast cells, and dilation of blood vessels with enlarged endothelial cells in the dermis, followed later by extensive necrosis of the auricle. Both UVA and UVB induced degeneration and necrosis of the epidermis. UVB rapidly produced more severe changes in the epidermis and dermis than UVA, a finding thought to be due to the higher energy of UVB light. Although the infiltration of lymphocytes and monocytes rather than neutrophils after irradiation with UVA and UVB, respectively, has been described in human skin, these were not seen in the mice of the present study.

Although the chromophore which initiates inflammation is unclear, reactive oxygen species (ROSs) and lipid peroxide are thought to be involved in UV-induced skin changes. These are associated with the breakdown of the native antioxidant protection system, which in the skin includes glutathione, catalase, and superoxide dismutase (SOD). UV irradiation damages many kinds of cells and constituents in the epidermis and dermis. Epidermal keratinocytes have been reported to release histamine, cyclooxygenase- and lipoxygenase-derived products of arachidonic acid, and various cytokines. In the present study, epidermal cells were injured by both UVA and UVB, and the difference in the dermal changes by these irradiations is of interest. In the dermis, fibroblasts have been described as accessible target for UVA and endothelial cells for UVB. UVA irradiation was shown in vitro to stimulate the synthesis and release of interleukin-6 from human fibroblasts, which induces the release of collagenase through an autocrine mechanism. In the present study, however, no apparent change in fibroblasts was observed simultaneously with neutrophil infiltration in the early phase after UVA irradiation. This may mean that neutrophils migrate out of blood vessels in response to damage to fibroblasts or other cells undetectable by light microscopy. On the other hand, UVB has been reported to stimulate endothelial cells to secrete various cytokines and chemical mediators and express the adherent molecules for inflammatory cells. Moreover, activated mast
cells themselves release free radicals as well as prostaglandin D₂ and leukotrienes. In the present study, it is possible that UVB irradiation targeted vascular endothelial cells and mast cells, which contributed to the mobilization of neutrophils and induction of edema, respectively, in the early stage of inflammation. The difference in histological findings between UVA and UVB suggests that chemical mediators inducing hyperpermeability of blood vessels are involved in the inflammation induced by UVB, but not that by UVA. However, it is widely thought that the generation of ROS initiates the above early responses induced by both UVA and UVB. Further studies are necessary to clarify the location of ROS generation in the auricle skin of mice.

With regard to the UVA-induced retinal degeneration, the photoreceptor, particularly the outer segment, has been reported to be the initial site of damage. Vesiculation, shortening and disorganization of the segment have been demonstrated in mice and rhesus monkeys after UVA irradiation at low levels with vacuolation or necrosis of the inner segment also observed at high levels. Furthermore, UVA irradiation at a higher level or for a longer period was shown to cause thinning or loss of the POS and thinning of the ONL. Corresponding with these reports, all the above UVA-induced changes were sequentially shown in our mice, and, in addition to vesiculation, an edematous appearance of the POS was also seen as an early change.

RPE cells continually phagocytose the packet of older discs shed from the POS through a continual renewal process. Therefore, accumulation of disrupted lamellar discs and lysosomal inclusion bodies in RPE cells with damaged POS has been reported in the retinal degeneration induced by many kinds of compounds and light irradiation. Furthermore, RPE cells are thought to invade into the photoreceptor segments. Corresponding to these changes, we also saw swelling of RPE cells and an increase in the number of migrating cells in the POS simultaneously with the POS change by UVA irradiation. The migrating cells in this study could have been derived from RPE cells. Interestingly, like UVA, UVB induced significantly increased number of migrating cells in the POS in the later stage, even though a markedly lower incidence of retinal occurred changes occurred than UVA. This may suggest that UVB induces very mild damage in the photoreceptors of animals.

ROSs have been generally implicated in contributing to the pathogenesis of retinal degeneration. The retina is richly supplied with oxygen from the choroidal plexus and the retinal vasculature and usually receives light stimulation which induces ROS generation in the photoactivation process. Additionally, the POS membrane is rich in polyunsaturated lipids and easily shows lipid peroxidation. UV light-induced damage is thought to be initially mediated through the enzymatic or physical impairment of mitochondria in the photoreceptors. The retinal degeneration observed in the present study is also thought to have been induced through this mechanism.

The present study clearly showed that changes in eye structures other than the retina were induced only by UVB irradiation. Corneal cell degeneration was initially observed, followed by lens epithelial degeneration (cortical cataract). These changes are similar to those documented in previous reports. On UVB irradiation, corneal squamus cells have been reported to be prematurely sloughed into the tear layers and cell division in the basal layer to be inhibited, followed by increased mitotic activity later. Chronic irradiation with UV light induced vascularization in the corneal stroma. Additionally, albino mice irradiated with near-UV light for a long period showed cortical cataract consisting of pyknotic nuclei in lens bow cells, indicating inhibited differentiation of the lens epithelial cells to fiber cells. These changes are also thought to be caused by ROS. Furthermore, the synergistic role of UV light with the thermal effect is thought to be important: lens temperature was shown to be elevated by 4.5°C in rabbits exposed to sunlight.

In the present study, UVA and UVB irradiations caused clearly different histological changes in the auricle skin and retina of albino Balb/c mouse.

References

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