Note

Measurement of Retinoids and β-Carotene 15,15'-Dioxygenase Activity in HR-1 Hairless Mouse Skin with UV Exposure

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Summary We investigated the vitamin A status and β-carotene 15,15'-dioxygenase activity in hairless mice with UV exposure to assess the regulation of vitamin A metabolism after UV irradiation. HR-1 hairless mice were irradiated with UV at 3 J/cm² for 5 d. After UV irradiation, the mice were sacrificed and samples were obtained to analyze the retinoid concentration, expression of RXR-α, and β-carotene 15,15'-dioxygenase activity. After UV exposure, the skin retinoid concentration was significantly lower as well as the expression of RXR-α. Higher skin β-carotene dioxygenase activity was observed in the UV group as compared to the control group. We found no significant differences in the α-tocopherol concentration or acrolein levels in the skins of the two groups. In conclusion, the elevation of β-carotene 15,15'-dioxygenase activity in hairless mice after UV exposure may be a response to reduction of the skin retinoid concentration.

Key Words UV exposure, hairless mouse, retinoids, α-tocopherol, β-carotene 15,15'-dioxygenase

Acute and chronic exposure to UV light leads to a variety of changes in the epidermis, dermis, and deeper parts of the skin. Not only does UV exposure lead to premature aging of the skin (photo-aging) and UV-induced hyperkeratosis or atrophy, but there is also enhancement of skin diseases, precancerous lesions, and skin tumors such as squamous cell carcinoma, basal cell carcinoma, and possibly malignant melanoma (1–3). Previous investigators have shown that exposure to UV light decreases the vitamin A content in hairless mice (4), and increases the activity of acyl CoA: retinol acyltransferase, an enzyme catalyzing the esterification of retinol. In humans, sunlight exposure is reported to decrease the β-carotene concentration of both plasma and skin (5), but changes in vitamin A metabolism and regulation after UV exposure have not been clarified yet.

β-Carotene 15,15'-dioxygenase (EC 1.13.11.21) is an enzyme which converts β-carotene to retinal (6, 7). Dietary intake of vitamin A, β-carotene, and fat is known to affect intestinal β-carotene dioxygenase activity (8–10). We previously reported a reduction of hepatic β-carotene dioxygenase activity in rats with streptozotocin-induced diabetes, which showed low plasma and high hepatic concentrations of retinol (11). However, little is known about dioxygenase activity in the skin. In this study, we measured vitamin A levels and β-carotene 15,15'-dioxygenase activity in UV-irradiated hairless mice to investigate the relationship between UV irradiation and the conversion of β-carotene to retinol.

Materials and Methods

Animals. Sixteen male hairless mice HR-1 (4 wk-old) were obtained from the animal center and housed in plastic cages in a well-ventilated room. After 2 wk on a standard diet (containing 10,000 IU of vitamin A per kg) and free access to water, the animals were divided into two groups of eight rats with UV-irradiated mice (UV group) and eight age-matched control mice (control group). This study was approved by the Animal Care and Use Committee of Osaka Medical College.

UV irradiation. As the UV source, four Toshiba FL-15 BL-B fluorescent lamps (peak wavelength: 352 nm, range: 300–430 nm) were used. Mice were placed in plastic cages with the floor 20 cm below the lamps for UV exposure. The amount of irradiation, measured at 360 nm by a UVR-2 UV meter (Topcon Ltd., Tokyo, Japan), was 3.0 J/cm² for 15 min per day. UV exposure was done for 5 d and then the mice were sacrificed after the final exposure.

Sample collection. After being sacrificed, samples of blood, liver, and skin from the back were obtained. Because a large amount of skin was needed for the analysis of β-carotene 15,15'-dioxygenase activity, eight of the 16 mice were used for the measurement of enzyme activity, while plasma, liver and skin for vitamin A analysis were obtained from the other eight mice.

Plasma was stored at −70°C with protection from light until analysis. Liver tissue and skin were homoge-
nized at 0°C in 50 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl] ethanesulfonic acid-KOH buffer (pH 7.4), containing 1.15% (w/v) KCl, 1 mM ethylenediaminetetraacetic acid, and 0.1 mM dithiothreitol (DTT). For the assay of β-carotene 15,15'-dioxygenase activity, the post mitochondrial supernatant was harvested after centrifugation at 10,000×g for 30 min.

Analysis of retinoids. Retinoid concentrations in plasma and liver tissue were assayed by HPLC (12). To measure free retinol concentration in the liver, the supernatant was extracted without saponification. The protein concentrations of the homogenate and the enzyme preparations were determined by the method of Bradford (13) using bovine serum albumin as the standard. Measurement of retinoid levels in the skin was done by the following method (14). Skin samples (about 1 g) were minced with scissors and homogenized after the addition of 350 μL of 50 mM acetate buffer (pH 4) and 1.5 mL of isopropanol/tetrahydrofuran (1:1) containing 200 μM butylated hydroxytoluene. Four milliliters of hexane were added and the mixture was vortexed for 30 s before being centrifuged for 5 min at 1,000×g. Then 3 mL of the upper hexane layer was harvested, transferred to another glass tube, and evaporated to dryness. The residue was dissolved in ethanol, and then injected into the HPLC system.

Analysis of α-tocopherol. The α-tocopherol concentrations were measured using HPLC with electrochemical detection, as described previously (15). HPLC was done with an Irika 871 pump (Irika Co., Ltd., Kyoto, Japan), an Irika 875 amperometric detector and Irika RP-18 column.

Measurement of β-carotene 15,15'-dioxygenase activity. β-Carotene 15,15'-dioxygenase activity was measured by the method of During et al. (16). The standard reaction mixture for the assay contained 15 μM all-trans β-carotene, 0.1 mM Tricine-KOH buffer (pH 8.0), 0.5 mM DTT, 0.15% Tween 40, 4 mM sodium cholate, 0.1 mM α-tocopherol, 15 mM nicotinamide, and an enzyme sample in a total volume of 0.2 mL. The reaction was conducted at 37°C for 30 min and was terminated by adding 50 μL of 37% (w/w) formaldehyde. Then, the mixture was incubated at 37°C for another 10 min. After acetonitrile (500 μL) was added, the mixture was mixed thoroughly, placed on ice for at least 5 min, and then centrifuged at 10,000×g at 4°C for 10 min. The supernatant was directly injected into the HPLC apparatus to measure the retinal concentration (16).

Assay of acrolein-lysine adduct. The supernatant of skin homogenate was stored frozen at -80°C until assay. The supernatants were applied for the determination of acrolein-lysine adduct by a competitive ELISA using a ACR-Lysine Adduct ELISA kit (NOF Co., Tokyo, Japan).

Western blotting analysis. Western blotting was done by a previously published method (17). Equal amounts (50 μg) of nuclear protein from the skin and recombinant retinoid receptor standards were used to determine the retinoid receptor levels with subtype specific anti-retinoid receptor antibodies (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA).

Statistical analysis. Results are expressed as the mean±SD, and the significance of differences was estimated by the Mann-Whitney U-test.

**Results**

The mean weight of the mice at the time of sacrifice was 26.0±1.2 g in the control group and 24.3±1.0 g in the UV group (p=0.058). The retinoid concentrations in the plasma, liver, and skin are shown in Table 1. The skin retinoid concentrations in the UV group (both retinol and retinyl palmitate) were significantly lower than in the control group, but there was no significant difference in the plasma retinol concentration or hepatic retinoid level between the two groups. Figure 1 shows RXR-α expression in the skin analyzed by Western blotting. In the UV group, the expression of RXR-α was decreased as compared to that in the control group. We also measured β-carotene 15,15'-dioxygenase activity in mice with and without UV exposure, and found higher enzyme activity in the skin of the UV group as compared to the control group (Fig. 2A). In contrast, there was no significant difference in hepatic dioxygenase activity (Fig. 2B). There were no histological differences between the groups with and without UV irradiation (data not shown). The skin α-tocopherol level was significantly decreased in the UV group compared to the control group (p<0.01).

**Table 1. Retinoid concentrations in hairless mice after UV exposure.**

<table>
<thead>
<tr>
<th></th>
<th>Control (n=4)</th>
<th>UV group (n=4)</th>
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<tbody>
<tr>
<td>Plasma retinol (mg/L)</td>
<td>0.594±0.171</td>
<td>0.507±0.210</td>
</tr>
<tr>
<td>Liver retinol (μg/mg·protein)</td>
<td>6.8±3.8</td>
<td>7.8±2.1</td>
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<tr>
<td>Liver retinyl palmitate (μg/mg·protein)</td>
<td>82.4±27.8</td>
<td>100.9±19.0</td>
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<tr>
<td>Skin retinol (ng/g·skin)</td>
<td>64.6±10.8</td>
<td>40.8±7.5*</td>
</tr>
<tr>
<td>Skin retinyl palmitate (ng/g·skin)</td>
<td>93.3±22.8</td>
<td>28.1±12.3**</td>
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Values represent the mean±SD.

* p<0.05, ** p<0.01.

Fig. 1. Expression of RXR-α in the skin shown by Western blotting. RXR-α was decreased in the mice with UV exposure.
decrease of both retinol and retinyl palmitate in the skin, plasma, and liver after UV exposure. There was a significant difference between two groups for either α-tocopherol or acrolein-lysine adducts. We found no significant differences between the groups with and without UV exposure. Thus, β-carotene dioxygenase activity might respond sensitively to alterations in the retinoid content in each tissue, as reported previously for streptozotocin-induced diabetic rats (11). Another factor that regulates the dioxygenase activity was reported to be free radicals. Lipid peroxidation suppresses the conversion of β-carotene to retinal (21) and α-tocopherol increases the central cleavage of β-carotene (22). UV exposure causes free radical generation in the skin (23) and alters its vitamin E content (24, 25). Acrolein is a product of overheated organic matter, incomplete combustion of plastic materials, cigarette smoking, and overheating frying oils, and is also reported as a marker of oxidative stress and long-term damage to protein in aging, atherosclerosis, and diabetes (26). In the present study, we found no significant differences in the skin concentrations of acrolein and α-tocopherol. This data suggests that free radicals may not be involved in the alteration of dioxygenase activity during UV exposure.

In conclusion, we demonstrated the elevation of β-carotene 15,15'-dioxygenase activity in hairless mice after UV exposure in response to a reduction of the skin vitamin A concentration. Further studies are required to clarify the regulation of skin retinoid, including the expression of β-carotene 15,15'-dioxygenase and the role of free radicals.

REFERENCES


