紫外線照射がヘアレスマウスの免疫器官および内分泌器官に及ぼす影響

Effects of Ultraviolet Irradiation on the Histological Changes in Immune and Endocrine Organs of Hairless Mice

数戸友子　近森絵令　益田敦子　小林佳代
村松加奈子　米田守宏　若村智子　Yi Li
登倉尋実　大石正

SUDO Tomoko[1]　CHIKAMORI Eri[1]　MASUDA Atsuko[1]　KOBYASHI Kayo[2]
MURAMATSU Kanako[3]　YONEDA Morihiro[4]　WAKAMURA Tomoko[5]　YI Li[6]
TOKURA Hiromi[6]　OISHI Tadashi[2*]

Effects of ultraviolet light (UV) irradiation on the skin physiology have been studied well. However, other effects than the skin physiology have not been investigated so much. Therefore, in this study, we examined the effects of UV irradiation on the immune and endocrine organs in hairless mice. Female hairless mice (Hr-/Kud; 8 weeks old) were used as a material. An artificial solar lamp (VITA-LITE; Light Sources Co. Ltd.) that simulates solar spectra including UV was used as a light source and a sheet of film (Acryplen: Mitsubishi Rayon) was used to remove UV. The animals were kept in a light and temperature controlled room (12 hour light and 12 hour darkness at 25℃). Significantly greater cyclobutane pyrimidine dimmers (CPDs) were detected in the skin of hairless mice when exposed to the solar simulated light with UV radiation (VITA-LITE) than that of animals exposed to the solar simulated light without UV. Organ weights (thymus, spleen, ovary, adrenal gland and liver) did not show statistical differences between the animals exposed to the solar simulated light with and without UV. Histological observation of these organs has not shown differences between the animals exposed to the solar simulated radiation with and without UV.

Thus, the intensity of UV radiation used in the present study was strong enough to induce DNA damage in the skin, but it did not induce histological changes in immune and endocrine organs.

Key Words: Solar simulated light, Ultraviolet radiation, Hairless mice, Immune organs, Endocrine organs

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1 Faculty of Science, 2 Graduate School of Humanities and Sciences, 3 KYOUSEI Science Center for Life and Nature and 4 Faculty of Human Life and Environment, Nara Women's University, Nara 630-8506, Japan, 5 Faculty of Medicine, Kyoto University, Kyoto 606-8501, Japan, 6 Institute of Textiles & Clothing, The Hong Kong Polytechnic University, Kowloon, Hong Kong, China

* Present Address: Nara Saho College, Rokuyaon-cho 806, Nara 630-8566, Japan (toishi@narasaho-c.ac.jp)

Introduction

Solar light consists of infra-red, visible and ultra-violet (UV) radiation. UV radiation is further divided into three regions; UVA (320-400 nm), UVB (280-320 nm) and UVC (200-280 nm). UVB and UVC are known to have bactericidal effects. In humans, UVB is responsible for sunburn and suntan, and induces skin cancer under excess exposure (Yoshikawa et al., 1990; Granstein and Matsui, 2004). Even low (suberythemal) doses of UVB were reported to cause reduction of the number of epidermal Langerhans cells which derived from the bone marrow and distribute in the skin to have immunological function (Ishitsuka et al., 2003; MacLoone et al., 2005). Chronic exposure to UVA radiation also induced reduction of Langerhans cells (Bestak and Halliday, 1996). Shen et al. (1999) reported that UVA radiation induced up-regulation of interleukin 12 and interferon γ that are integumentum helper T cell cytokines. Therefore, UV irradiation may induce effects on blood structure as well as immune and endocrine organs through the skin. In the present study, we tried to investigate the effects of artificial solar simulated radiation with UV and without UV on the DNA damage in the skin and on the histology of immune and endocrine organs.

Materials and Methods

Fifteen hairless mice (Hr-/Kud, 8 week-old females) with JC1:ICR (Japan Clea Co. Ltd.) background were used as a material. White fluorescent lamps (FLS40S/EX-N/MX 40W, Matsushita Co. Ltd.) and solar simulated lamps (VITA-LITE; 3826EX-LS 40W, Light Sources Co. Ltd.) were used as light sources. A UV cut filter (Acryplen; HBS 001-50 μm; Mitsubishi Rayon Co. Ltd.) was used as a filter to remove UV from the solar simulated light. Animals were placed at about 30cm from the light sources. Light intensity (illuminance) was measured with Digital luminometer (T-10: Minolta Co. Ltd.). UVA was measured with UV Monitor MS-211-I (Eiko Seiki Co. Ltd.). The total amount of UV (UVA + UVB) was measured with UV-340 (Sato Shoji Co. Ltd). The intensities were 3500-4800lx, UVA:12-90J/m^2/12h and UV:16-30 μ W/cm^2 for the VITA-LITE group, and 4600-5800lx, UVA: 0-12J/m^2/12h and UV:6-10 μ W/cm^2 for the Acryplen group. The animals were raised under 12 hour light and 12 hour darkness (LD 12:12; light on at 6:00 and light off at 18:00) in temperature controlled room at 25℃. The animals were divided into 3 groups (Start, VITA-LITA (solar simulated light with UV), VITA-LITE with Acryplen (solar simulated light without UV). The animals were kept under white fluorescent lamps without UV radiation before the start of the experiment. Telemeters (XM-FH, Mini-Mitter Co. Inc.) were implanted into the abdomen of mice to measure body temperature and locomotor activity. Implantation was done under anesthesia and then, they were raised in individual cages. Recording and analyses of body temperature and locomotor activity rhythms were done by Vital View telemeter system (Mini-Mitter Co. Inc.). Twenty-three days after the start of the experiment, animals were killed and the blood was taken from the heart. The numbers of red and white blood cells were counted, and the percentages of segmented form of neutrophils (SEG), lymphocytes (LYMPH) and monocytes (MONO) were obtained. The amount of hemoglobin (Hb), hematocrit value (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were also obtained. The eyes, thyroid gland, thymus, spleen, adrenal gland, ovary were dissected out, and fixed in Bouin's solution after measuring the weights. Paraffin sections were made and they were stained with hematoxylene and eosin. A piece of skin (1cm × 1cm) was taken to measure the amount of cyclobutane pyrimidine dimmers (CPDs) by Enzyme-Linked Immunosolubent Assay (ELISA; Thermo Labsystems, Cat. No. 2801, Franklin, MA).

Statistical analyses were done by Tukey test.

Results

1. Body weight and the amount of food intake

Body weight and the amount of food intake were increased from the beginning of the experiment to the end of experiment in both groups (Fig. 1). However, there were no differences either in the body weight or in the amount of food intake between the VITA-LITE group (UV+) and the Acryplen group (UV-).

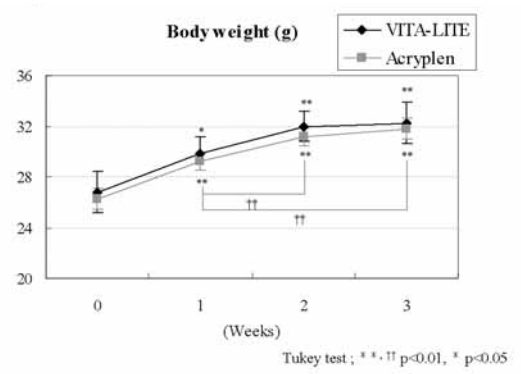

Fig. 1. Body weight changes of VITA-LITE group (UV+) and Acryplen group (UV-) during the experiment from the beginning to the end.

2. Organ weight

Organ weights were shown in Table 1. The thyroid was so small that the weight could not be measured. The liver and ovary weights were greater in the VITA-LITE and the Acryplen groups in comparison with the start group probably owing to growth of animals. The thymus weight was significantly greater in the Acryplen group (UV-) than in the start group, while the VITA-LITE group (UV+) did not show a significant increase. There were no differences between the VITA-LITE and Acryplen groups in the weights of the other organs.

3. Blood structure

The leukocyte structure (ratios of segmented form of neutrophils, monocytes and lymphocytes) showed no differences among the groups (Table 2). There were no differences in the number of red and white blood cells, the amount of hemoglobin or the hematoclit value among the groups. MCH (mean corpuscular hemoglobin) significantly reduced in the VITA-LITE group (UV+) comparing to the start group, while MCV and MCHC did

Table 1. Body weight and organ weights of the hairless mice at the beginning of the experiment (Group: Start) and after 23days of exposure to the solar simulated light with UV (Group: VITA-LITE) and the UV-removed solar simulated light (Group: Acryplen).

		Organ weight (g)						
group	Body weight (g)	Right eye	Left eye	Thymus	Liver	Spleen	Adenal gland	Ovary
Start	27.93±0.76	0.045±0.014	0.045±0.009	0.082±0.014	1.912±0.157	0.157±0.036	0.010±0.002	0.012±0.002
VITA-LITE	32.84±1.29	0.051±0.009	0.052±0.009	* 0.098±0.021	* 2.345±0.148 *	0.201±0.014	0.015±0.005	0.016±0.002 *
Acryplen	31.97±1.05	0.046±0.009	0.045±0.009	0.114±0.011	2.238±0.125	0.182±0.040	0.012±0.004	0.015±0.002

Tukey test ; ** p<0.01, * p<0.05

Table 2. Leukocyte structure and erythrocyte structure. WBC: white blood cell, SEG: segmented form of neutrophil, MONO: monocyte, LYMPH: lymphocyte, RBC: red blood cell, Hb: Hemoglobin, HT: hematoclit, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration

	Leukocyte structure				Erythrocyte structure					
	WBC	SEG	MONO	LYMPH	RBC	Hb	HT	MCV	MCH	MCHC
group	(/μL)	(%)	(%)	(%)	(x10^4/mm^3)	(g/dL)	(%)	(μ^3)	(pg)	(%)
Start	5160±2840	33.80±13.83	4.20.±3.27	62.00±14.92	778±103	14.08±1.74	48.58±7.39	62.40±1.82	18.00±0.71	29.20±1.79
VITA-LITE	5400±1668	33.20±11.71	1.60±1.14	65.20±11.23	821±56	13.94±0.95	50.74±4.12	61.80±1.92	16.80±0.45 *	27.60±1.52
Acryplen	5520±3030	32.80±8.23	0.80±0.84	66.40±8.99	744±60	12.76±0.71	45.04±4.40	60.40±1.34	17.20±0.45	28.20±1.30

Tukey test ; * p<0.05

not show any differences among the groups.

4. The skin

The amount of CPDs measured by ELISA was shown as the absorption value in Fig. 2. The value was significantly lower in the Acryplen group (UV-) than those of the start and VITA-LITE group (UV+). This indicates that the artificial solar simulated light without UV significantly reduced CPDs in comparison with that with UV.

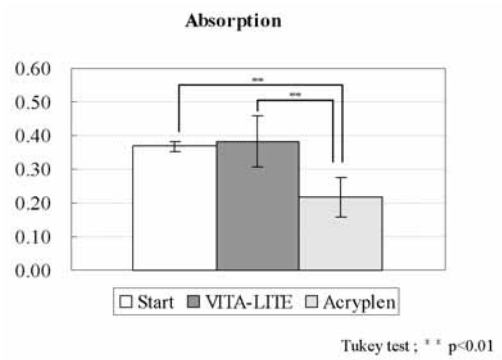

Fig. 2. Skin DNA damage (CPDs) expressed as absorption measured by ELISA. The value in the Acryplen group (UV-) is significantly less than those in the Start and the VITA-LITE group (UV+).

5. Histological observation (Fig. 3)

In the adrenal gland, there were many vacuoles

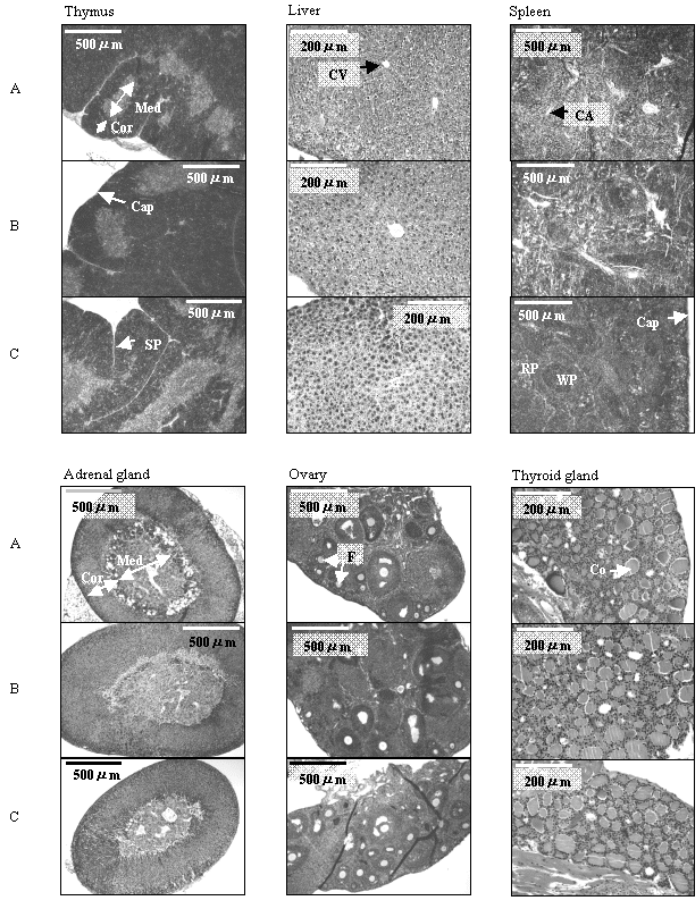

Fig. 3. Histology of immune and endocrine organs in the Start (A), VITA-LITE (B) and Acryplen (C) groups. In the thymus, Med: medulla, Cor: cortex, Cap: capsule, SP: septa. In the liver, CV: central vein. In the spleen, RP: red pulp, WP: white pulp, CA: central artery, Cap: capsule. In the adrenal gland, Cor: cortex, Med: medulla. In the ovary, F: follicle. In the thyroid gland, Co: colloid.

between the medulla and cortex in the start group, but these vacuoles disappeared in the VITA-LITE (UV+) and the Acryplen (UV-) groups. In the liver, the size of the hepatocytes seems to have enlarged in both VITA-LITE (UV+) and the Acryplen (UV-) groups in comparison with that of the start group, which may reflect the growth of the liver as shown in Table 1. However, UV exposure did not induce significant differences in the histology of any organs observed (the adrenal, liver, thymus, spleen, ovary and thyroid gland).

Discussion

Since the CPDs in the skin were significantly reduced in the Acryplen group (UV- solar simulated light) in comparison with those in the VITA-LITE group (UV+ solar simulated light), UV appears to be responsible for this difference. This is in accordance with the reports on the effects of UV on the DNA damages of the skin (Vink et al., 1994; Ichihashi and Sasaki, 2000). Since a significant reduction of Langerhans cells has been reported in UVA and UVB irradiated mice (Bestak and Halliday, 1996; Ishitsuka et al., 2003), the result in the thymus weight might reflect the effect of UV exposure. Reduction of MCH in the UV-exposed mice (Table 2) suggests that UV affects some blood structures. In the present study, the effects of UV were not so strongly exhibited probably because of low intensity of light used. Other possible reasons are that visible light was always irradiated throughout the experiment. Mulero et al. (2006) reported that oxidative stress-related markers such as glutathione S-transferase and glutathione peroxidase activity increased in erythrocytes of UV-exposed hairless rats. It would be necessary to expose the animals to only UV radiation such as black light. Since even low doses of UVB and UVA induced depletion of Langerhans cells in mice (Bestak and Halliday, 1996) and changes in blood immune systems in humans (Ullrich, 2005), it would be necessary to count the number of Langerhans cells and to measure the amount of cytokines in future experiments.

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紫外線照射がヘアレスマウスの免疫器官および内分泌器官に及ぼす影響

数戸友子　近森絵令　益田敦子　小林佳代
村松加奈子　米田守宏　若村智子　Yi Li
登倉尋実　大石正

紫外線が皮膚に及ぼす影響については，多くの研究がある．しかしながら，皮膚以外に及ぼす影響についての研究は少ない．そこで，本研究においては，ヘアレスマウスを用いて免疫器官および内分泌器官に及ぼす影響について組織学的に検討した．雌のヘアレスマウス（Hr-/Kud；8 週令）を材料として用いた．紫外線を含む太陽光を模した人工太陽光ランプ（VITA-LITE）を光源として用いた．また，紫外線を除去するために紫外線カットフィルム（アクリプレン；三菱レイヨン）を用いた．明暗周期（12 時間明：12 時間暗），恒温（25℃）の条件下で動物を飼育した．紫外線を含む人工太陽光に照射されたマウスは，アクリプレンフィルターで紫外線をカットしたマウスに比べてシクロブタン型ピリミジンダイマーが顕著に多かった．器官重量（胸腺，脾臓，卵巣，肝臓）は紫外線を含む人工太陽光と紫外線をカットした人工太陽光の間で違いは見られなかった．また，血液像，組織学的観察においても両者の間に顕著な違いは見られなかった．

キーワード：人工太陽光，紫外線，ヘアレスマウス，免疫器官，内分泌器官