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Skin morphology at the time of UV irradiation is important for wrinkle formation

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Synopsis

Hairless mice (HR/ICR) were irradiated chronically with suberythemal doses of UV-B radiation (ultraviolet radiation in wavelength 290–320 nm) immediately before and after production, with cyanoacrylate resin, of an artificial temporary groove parallel to the midline, which is an unusual direction for wrinkle formation. Data for UV radiation-exposed skin before or after production of an artificial groove and in chronological age-matched control mice or mice treated only by production of an artificial temporary groove were compared. Visible signs of artificial wrinkling were present after approximately six weeks of UV-B irradiation and were very apparent after ten weeks of irradiation. From image analysis of skin impressions after ten weeks, artificial wrinkling in skin treated by UV-B irradiation after production of an artificial groove was significantly increased compared with skin in which an artificial groove was produced after UV-B irradiation. These results indicated that both an artificial temporary groove in the skin and UV-B irradiation immediately after production of the temporary groove are necessary for wrinkle formation in this mouse model, and suggested that the skin morphology at the time of UV-B irradiation is important for wrinkle formation.

INTRODUCTION

Wrinkles are the most common of all the signs of aging. There have been many histological studies of wrinkles in the skin of aged human subjects (1–3). Wrinkles of facial expression occurring on the facial skin are the most well developed and become permanent with chronological aging (4–7). The facial skin responds to every movement of the underlying muscles in smiling, frowning, and physical movement, and such movement produces temporary but repeated wrinkling of the same portion of the face. This repeated temporary wrinkling has been suggested to play an important role in the formation of permanent wrinkles. Hairless mice have been used extensively in studies of the formation of wrinkles after chronic UV-B irradiation (ultraviolet radiation in wavelength range 290–320 nm) (8–12). Kiss *et al.* (11) reported that there are other factors in addition to total cumulative UV-B dose that are important for the appearance of wrinkling in this model. Despite the suggestion that repeated temporary wrinkling

plays an important role in the formation of permanent wrinkles, there have been few studies of the factors associated with wrinkle formation using hairless mice.

We previously evaluated the effects of temporary skin fixation on wrinkle formation using the back skin of hairless mice (13). In the group exposed for 20 weeks to UV-B irradiation immediately after production of the artificial groove parallel to the midline, a wrinkle formed at right angles to the groove, which is an unusual direction for wrinkle formation. Therefore, in this study, we examined the first ten-week period quantitatively, using image analysis to determine how early wrinkle formation can occur, and we also studied the effects of production of a temporary groove in the skin.

MATERIALS AND METHODS

ANIMALS

HR/ICR albino hairless mice were used in this study. This strain was derived by crossing hairless mice (HR/HR), originally obtained from Nisseiken Corporation, Japan, and the albino strain HaM/ICR. The HR/ICR strain represents a line maintained under conventional conditions in our laboratory for several generations of hairless brother/phenotypically normal haired sister mating. All experiments were performed with hairless female mice only, which had free access to food and water throughout the experimental period. Animals were housed in rooms where the lighting without UV emission was automatically regulated on a 12-hour light/dark cycle.

RADIATION SOURCE AND PRODUCTION OF ARTIFICIAL TEMPORARY GROOVE

The hairless mice were divided into five groups from the same stock at six weeks: group 1, UV-B irradiation immediately after production of the artificial temporary groove; group 2, production of the artificial temporary groove immediately after UV-B irradiation; group 3, production of the artificial temporary groove only (no irradiation); group 4, UV-B irradiation only; group 5, no treatment. Group 1 and 2 UV-B mice were placed in cages in groups of nine animals each and were irradiated by a bank of six Toshiba SE lamps with no filtering for UV-B. The distance from the lamps to the animals' backs was 35 cm. The animals were exposed to UV-B at a dose of 65 mJ/cm^2 (1 MED = 70 mJ/cm^2) five times weekly for ten weeks, yielding a total dose of 32.5 J/cm^2. The energy output of the lamps (at 35 cm) was measured with a Topcon Co. Ltd. UV-radiometer 305/365DII. The spectral irradiance of these lamps was measured with an Optical Science Co. Ltd. MSR7000 radiospectrometer, the spectral output of which is shown in Figure 1. The mice in the test groups were anesthetized with ether ten minutes before irradiation, and the back skin was fixed with cyanoacrylate resin to produce an artificial groove parallel to the midline (Figure 2). The control groups were not treated or were treated similarly but without UV irradiation. During the period of exposure, the animals could move around freely in their cages. The template (cyanoacrylate resin)-induced groove seen after completion of the UV irradiation protocol was confirmed to be restored to the previous state after one to two hours.

GRADING OF VISIBLE CHANGES

Skin wrinkling in hairless mice was assessed weekly as described by Bisett *et al.* (8), as

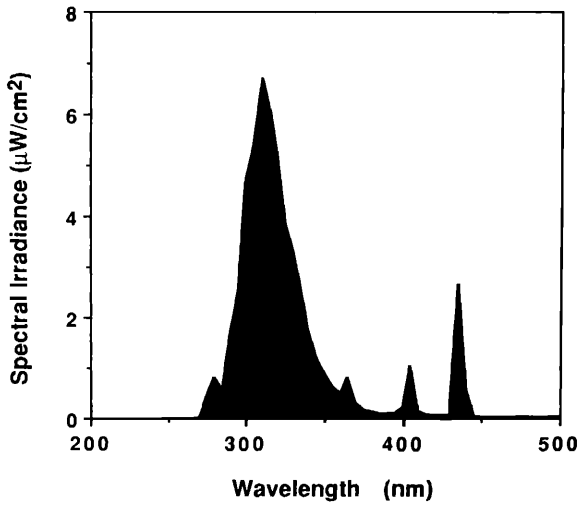

Figure 1. Spectral irradiance of the unfiltered SE lamps as measured with the radiospectrometer.

follows: grade 0, no coarse wrinkles; grade 1, a few shallow coarse wrinkles; grade 2, some coarse wrinkles; grade 3, several deep coarse wrinkles. The scale ranged from 0 for normal animals to 3 for the heavily wrinkled skin. For convenience in grading, mice were held by the tail with their feet resting against a solid surface to diminish movement.

WRINKLE MEASUREMENT

After ten weeks, impressions were made of the back skin of nine unrestrained mice, using EXAFINE hydrophilic vinyl polysiloxane impression material (GC Corp. Tokyo, Japan). We set the impression of wrinkles on the sample stand so that the measurement surface was horizontal, and produced wrinkle shadows by illumination with light of a fixed intensity at 30°, using a fiber optic light source (Nikkon). The shadow images were photographed with a still video camera (MS-C1100) and a digital image recorder (MS-R1100, Minolta) with a macro50 lens system and were input into an image analyzer (LA555 personal image analysis system, PIASS Co Ltd., Japan). Figure 3 shows examples of a shadow image and binary image obtained by extracting shaded areas of the image at a constant gray level. We measured the shadow area for all shadows in one image, using the image analyzer, and calculated the ratio of wrinkle area (%), defined as the ratio of the sum of the shadow area to the measured area.

STATISTICS

The wrinkle grading score and wrinkle area (%) were expressed as mean ± standard deviation. Differences between means were checked for significance using Student's *t*-test.

RESULTS

From grading of visible changes, original wrinkling on the back skin of hairless mice

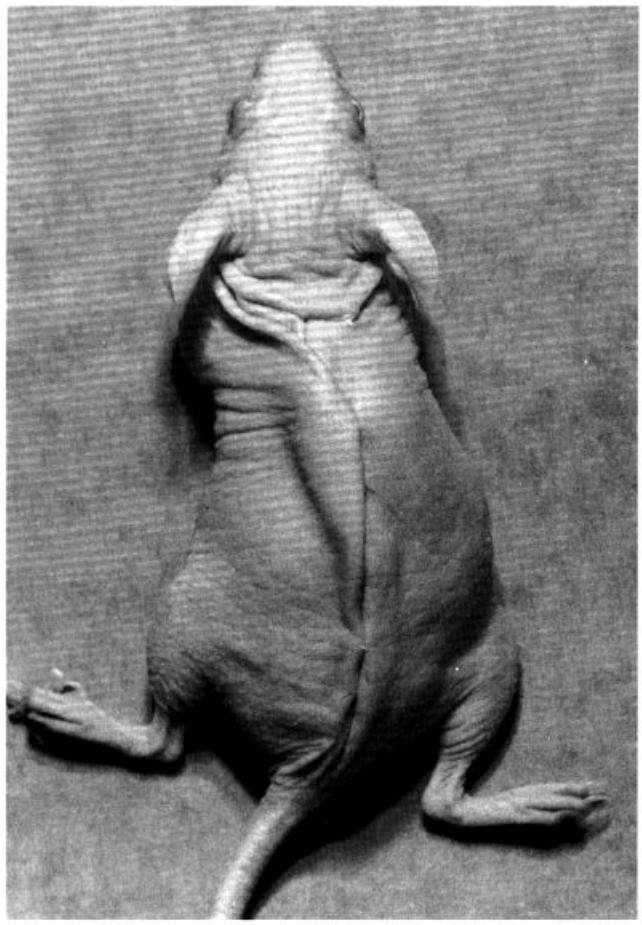

Figure 2. Photographs of mouse skin before irradiation. The back skin was fixed with cyanoacrylate resin to produce an artificial groove parallel to the midline.

after UV-B irradiation in the usual state was observed in groups treated by both UV-B irradiation immediately after production of the artificial groove and by production of an artificial temporary groove after UV-B irradiation. The degree of wrinkling in the latter group was greater than that in the former group and similar to that in the group treated only with UV-B irradiation (Figure 4). Visible signs of original wrinkling were present after approximately three weeks of UV-B irradiation and were very apparent after ten weeks.

On the other hand, artificial skin wrinkling parallel to the midline was only induced by UV-B irradiation immediately after production of an artificial temporary groove. No such wrinkling was induced by production of an artificial temporary groove after UV-B irradiation. Furthermore, no such changes were observed in the group in which the temporary groove alone was produced without UV-B irradiation (Figure 5). In the artificial wrinkle group, animals also developed wrinkles, but the onset was first observed at week six. In both original and artificial groups, wrinkles after ten weeks of UV-B irradiation did not disappear even when the skin was stretched, suggesting

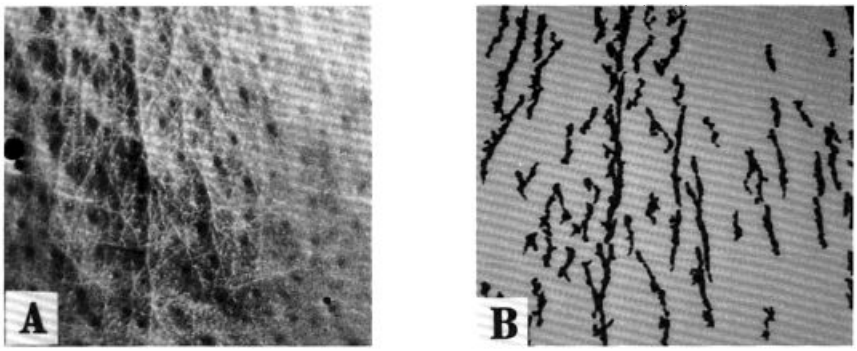

Figure 3. Measurement of wrinkles on skin impressions of the backs of hairless mice by image analysis. (A) Representative shadow image and (B) binary image obtained by extracting shadowed areas at a fixed gray level.

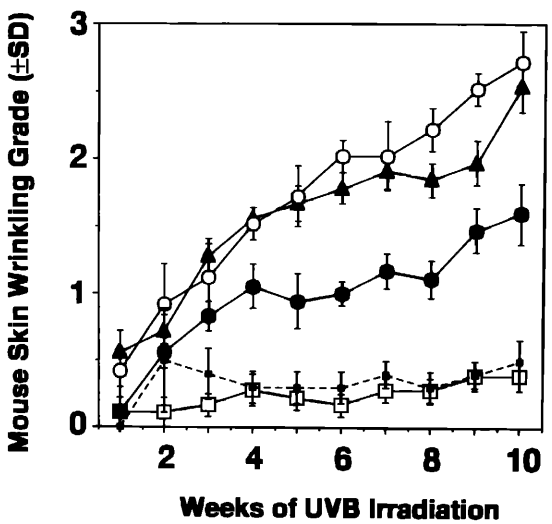

Figure 4. Effects of temporary artificial groove on wrinkle formation (original direction: at right angle to the midline) using the back skin of hairless mice. Closed circles: UV irradiation after production of temporary wrinkle. Open circles: UV-B irradiation only. Closed triangles: production of temporary wrinkle after UV-B irradiation. Open squares: production of temporary wrinkle without UV-B irradiation. Closed squares: no treatment. Error bars represent one standard deviation about the mean.

permanent wrinkles. The differences are further illustrated in Figures 6 and 7, in which the ten-weeks macro-photographs of animals and skin impressions of all groups are compared. As shown, artificial skin wrinkling parallel to the midline was only induced by UV-B irradiation immediately after production of an artificial temporary groove.

The degree ratios of wrinkling calculated by image analysis are shown in Table I. In the groups treated by UV-B irradiation after production of the temporary groove, by production of the temporary groove after UV-B irradiation, and by UV-B irradiation only, original wrinkling was significantly increased compared with age-matched controls and the group in which only the temporary groove was produced. In the groups treated by UV-B irradiation after production of the temporary groove and in those in which the

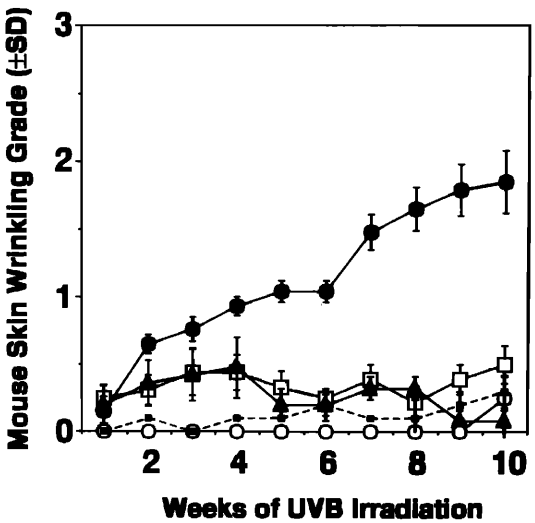

Figure 5. Effects of temporary artificial groove production on wrinkle formation (artificial direction: parallel to the midline) using the back skin of hairless mice. Closed circles: UV-B irradiation after production of temporary wrinkles. Open circles: UV-B irradiation only. Closed triangles: production of temporary wrinkles after UV-B irradiation. Open squares: production of temporary wrinkles without UV-B irradiation. Closed squares: no treatment. Error bars represent one standard deviation about the mean.

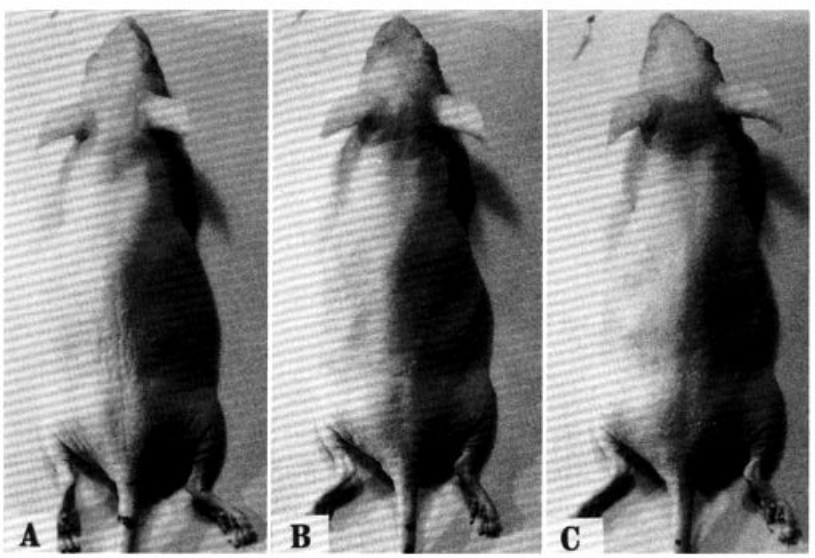

Figure 6. Photographs of mouse skin after ten weeks. (A) UV-B irradiation immediately after production of the artificial groove parallel to the midline. (B) Production of the artificial groove parallel to the midline immediately after UV-B irradiation. (C) Only the artificial groove was produced without UV-B irradiation.

temporary groove was produced after UV-B irradiation, original wrinkling was slightly but significantly decreased compared with the UV-B irradiation-only group. There were no differences in original wrinkling in the group treated only by production of the temporary groove compared with age-matched controls. In the groups treated by UV-B irradiation after production of the temporary groove, artificial wrinkling was signifi-

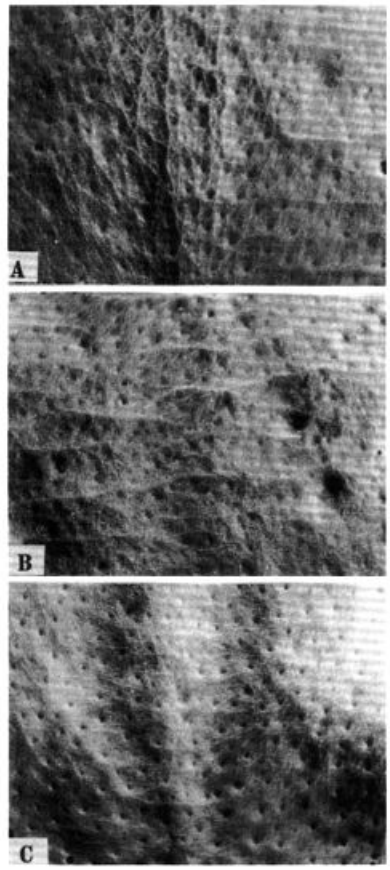

Figure 7. Photographs of skin impressions (replicas) after ten weeks. (A) UV-B irradiation immediately after production of the artificial groove parallel to the midline. (B) Production of the artificial groove parallel to the midline immediately after UV-B irradiation. (C) Only the artificial groove was produced without UV-B irradiation.

cantly increased compared with all other groups. There were no differences in artificial wrinkling in the group treated by production of the temporary groove after UV-B irradiation compared with the group treated by production of the temporary groove only. In the group in which only the temporary groove was produced, artificial wrinkling was significantly increased compared with age-matched controls.

DISCUSSION

We previously evaluated the effects of temporary skin fixation on wrinkle formation after 20 weeks of UV-B irradiation using the back skin of hairless mice, and suggested that both production of a temporary groove in the skin and UV-B irradiation are necessary for wrinkle formation (13). In this study, we examined the first ten-week period quantitatively to determine how early wrinkle formation can occur, and we also studied the different effects of production of a temporary groove. Our results confirm that both the artificial temporary groove in the skin and UV-B irradiation immediately after—but not

Table I
The Results of the Ratio of Wrinkle Calculated by Image Analysis

Treatment	Ratio of wrinkle (image analysis %) ± S.D.	
	Original[a]	Artificial[b]
Control	1.74 ± 0.80*,†	0.56 ± 0.43*,†,‡
UV-B only	21.68 ± 2.06*,†,‡	2.12 ± 1.32*,†,‡
Temporary groove only[c]	3.81 ± 1.89*,†	4.32 ± 1.30*
Temporary groove after UV-B[d]	14.05 ± 5.35‡	5.68 ± 3.70*
UV-B after temporary groove[e]	16.91 ± 4.58‡	13.74 ± 4.07*,‡

* Significantly different from the group characterized by production of temporary groove after UV-B irradiation ($p < 0.01$).
† Significantly different from the group characterized by UV-B irradiation after production of temporary wrinkle ($p < 0.01$–0.05).
‡ Significantly different from the group characterized only by production of temporary wrinkle ($p < 0.01$–0.05).
[a] Original wrinkle at right angles with the midline, which was observed in usual state.
[b] Artificial wrinkle parallel to the midline, which was unusual direction of wrinkle formation.
[c] Group characterized by production of temporary wrinkle without UV-B irradiation.
[d] Group characterized by production of temporary wrinkle after UV-B irradiation.
[e] Group characterized by UV-B irradiation after production of temporary wrinkle.

before—production of the temporary groove are necessary for wrinkle formation in this mouse model, and we suggest that the skin morphology at the time of UV-B irradiation is important for wrinkle formation.

Recently, wrinkle development in these mice in response to irradiation has been taken to be an indicator of chronic UV light exposure, or photoaging (4–7). Bissett *et al.* (8) reported slight wrinkle formation after five weeks of UV-B irradiation and permanent wrinkles after about 15 weeks. Kiss *et al.* (11) investigated the effects of high- and low-dose UV-B exposure on the degree of hairless mouse skin wrinkling and observed that wrinkle formation does not parallel the UV-B irradiation dose. Moloney *et al.* (12) reported that visible signs of wrinkling were present after approximately six weeks of UV-B irradiation and were very apparent after ten weeks of irradiation. The direction of wrinkle formation observed in our study differed from that in the above studies. However, the timing of the initiation of wrinkles seemed to be similar. This observation may suggest that the total cumulative dose is important for the timing of the initiation of wrinkles.

Previously, we considered two possible mechanisms for wrinkle formation after temporary fixation. Tissue destruction and subsequent tissue reconstruction occur in the areas exposed to UV-B but not in other areas, and this may cause distortion in the tissue, resulting in wrinkle fixation. Alternatively, when reconstruction after destruction is continuously accelerated in the entire tissue exposed to UV-B, tissue reconstruction may proceed along the temporary groove, causing fixation of the wrinkle. In our experimental system, the temporary groove was confirmed to be restored to the previous state after one to two hours. In this study, no artificial wrinkling was induced by production of an artificial temporary groove after UV-B irradiation, and these findings suggest that the latter hypothesis is unlikely. Therefore, the primary cause of wrinkle formation after fixation followed by UV-B irradiation may be differences in local changes due to uneven

UV-B irradiation. On the other hand, our results show a slight delay in the formation of longitudinal wrinkles after temporary fixation, compared with the formation of original transverse wrinkles. Thus, there remains a slight possibility that the latter hypothesis described above may explain wrinkle formation.

The connective tissue elements collagen and elastin are known to be degraded by collagenase and elastase produced by inflammatory cells (14,15) and fibroblasts (16,17). Collagenase mRNA levels in the dermis (18) and cultured fibroblasts (19) were reported to increase after UV-A irradiation. It is well known that exposure of keratinocytes in culture (20) or of the skin *in vivo* (21,22) to UV irradiation induces marked elevation of release of several cytokines, including interleukin-1 (IL-1) and tumor necrosis factor (TNF). UV-inducible IL-1 has also been shown to stimulate the synthesis of elastase (23) and to increase collagenase activity (24) in normal human fibroblasts. On the other hand, recent studies have shown up-regulation of the elastin mRNA level by IL-1β (25) and its down-regulation by TNF (26). Thus, it is conceivable that the synthesis and degradation of connective tissue elements are regulated by cytokines. In addition, recent studies have shown the expression of collagenase by normal human fibroblasts in response to singlet oxygen radicals generated by UV-A irradiation (27,28). Active oxygen species induce cross-linkage of collagen *in vitro* (29) and *in vivo* (30). Oxidative autoactivation of latent collagenase by human neutrophils has also been reported (32). Application of a radical scavenger has been reported to inhibit wrinkle formation in animals (31). Considering the expression of proteins such as collagenase, there may be a time lag between UV-B exposure and expression of enzyme activity, supporting the uneven irradiation theory. However, cross-linking due to active oxygen species and activation of latent-type collagenase (32) suggests that the skin morphology at the time of UV-B irradiation is important for wrinkle formation. Although it is unclear which mechanism is involved, wrinkles can be formed in any direction by temporary fixation followed by UV-B irradiation. Further studies are needed to determine whether this phenomenon occurs in other animal species and whether uneven irradiation, such as that by slit rays, is also the same effect.

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