The skin injury induced by high energy dose of ultraviolet in hairless descendants of Mexican hairless dogs

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**Summary.** Histopathological changes in the dorsal skin of hairless descendants of Mexican hairless dogs (MHDs) exposed to artificial irradiation with high energy dose (180kJ/m \(^{2}\)) of ultraviolet (UV) rays (UVA+B) were investigated.

Macroscopically, erythema and edema were observed in the irradiated skin at 1 day after irradiation (DAI), and blister formation occurred except one dog at 2 DAI. Erythema almost disappeared at 5 DAI, and at 6 DAI, the skin recovered to almost normal state. Light microscopically, sunburn cells were observed at 1 DAI. Then intercellular edema and blister formation in the epidermis and dermal edema were evident at 2 and 3 DAI. At 6 DAI, the skin showed almost normal features except for slight epidermal thickening, but melanin granules, which were distributed in almost the whole length of the epidermis before UV irradiation, were detected only in cells which seemed to be melanocytes except one dog. Dihydroxyphenylalanine (DOPA)-positive melanocytes almost disappeared at 1 and 2 DAI, and at 6 DAI, the number of DOPA-positive melanocytes increased over the level before UV irradiation. The ultrastructural features of melanocytes were characterized by vacuolated cytoplasm, decreased melanosomes, irregular-shaped nuclei and shortened dendrites at 1 DAI, and returned to normal at 6 DAI. These findings of melanocytes reflect the severity of the skin injury and support weak suntan reaction in this case. In conclusion, severe form of UV-induced skin injury seen in humans could be reproduced in hairless descendants of MHDs exposed to high energy dose of artificial UVA+B.

**Key words:** Melanocyte, Mexican hairless dog, Skin, Ultraviolet ray

**Introduction**

In 1983, a pair of Mexican hairless dogs (MHDs) was introduced into the Research Center, Nihon Nosan Kogyo Co., Ltd., Tsukuba, Japan, and an autosomal dominant gene (Hm: hairless, Mexican type) responsible for the hairless characteristics of MHD has been identified (Kimura et al., 1993).

Now, a colony of hairless descendants between a male MHD and female beagles is maintained. Those hairless dogs have abundant melanocytes in the epidermis (Ishii et al., 1995) and develop melanin deposition in response to solar ultraviolet (UV) irradiation (Kimura and Doi, 1994a). In this connection, most of hairless animals previously reported have no melanocytes which produce melanin granules (Rigdon and Packchanian, 1974; Hanada et al., 1988; Sundberg et al., 1989). Therefore, the hairless dogs are expected to become a useful model for investigation of the effects of UV-irradiation on human skin.

On the other hand, it is easily imagined that skin responses to UV irradiation are influenced both by kind of UV light source and by energy dose of UV. Recently, Kimura and Doi (1995) compared histopathological skin reactions of hairless descendants of MHDs between solar exposure and artificial UV irradiation (low energy dose), and they reported that solar exposure (90kJ/m \(^{2}\)) provoked more remarkable pigmentation while artificial UV irradiation (40kJ/m \(^{2}\)) brought about more prominent sunburn reactions in the dorsal skin.

The purpose of this study is to clarify the skin reactions light and electron microscopically in hairless descendants of MHDs exposed to artificial irradiation with high energy dose (180kJ/m \(^{2}\)) of UV.

**Materials and methods**

Three 1-year-old male hairless descendants of MHDs were used. Dogs were individually housed in stainless steel cages (90x90x90 cm) in an animal room.
under controlled conditions (temperature: 25±2 °C; 
relative humidity: 50±10%; air exchange: 10 to 15 times 
per hour; light cycle: 12-hour light and 12-hour dark). 
They were fed a commercial dry dog food (Labo D 
standard, Nihon Nosan Kogyo Co. Ltd., Yokohama, 
Japan) and water ad libitum.

Dogs were irradiated with artificial UV irradiator 
HP-30BLB, which has a peak in UVA region (365nm) 
and HP-30M, which has a peak in UVB region (312nm) 
(ATTO Co. Ltd., Tokyo, Japan) for two hours. During 
irradiation, each dog was placed in a wire cage 
(85x95x75cm) with 2 cm mesh. The irradiated sites 
(10x10 cm) were confirmed to the skin over the dorsum 
of each dog. A total irradiation dose for two hours was 
approximately 180kJ/m².

In order to evaluate the erythematos change in the 
irradiated skin by recording the skin color (redness), a 
spectrophotometer (CR-200, Minolta Co. Ltd., Tokyo, 
Japan) was used. Recording was done at one day before 
UV irradiation, and at 1, 2, 3, 4, 5 and 6 days (DAI) and 
2 and 4 weeks (WAI) after UV irradiation, respectively.

After macroscopic examinations, skin samples were 
obtained from 2 portions of the dorsal skin of each dog 
using a 6-mm biopsy punch (Nagatoi Co. Ltd., Tokyo, 
Japan) under local anesthesia with 2% lidocaine at one 
day before UV irradiation and at 1, 2, 3, 4, 5 and 6 DAI and 
2 and 4, 8 and 12 WAI, respectively.

Half of the skin samples were rinsed in 0.1M 
phosphate buffer (PB; pH 7.4) and incubated in 2N 
Sodium bromide for 2 hours at 37 °C in order to separate 
the epidermis from the dermis. The separated epidermal 
sheets were fixed in 10% cold neutral buffered formalin 
for 30 min, washed twice with 0.1M PB, and incubated in 
3.1mM dihydroxyphenylalanine (DOPA) in 0.1M PB 
for 5 hours. DOPA-positive melanocytes in the 
epidermal sheets were observed under light microscope.

The remaining skin samples were used for light 
and electron microscopic examinations. For light 
microscopic examinations, the skin specimens were 
fixed in 10% neutral buffered formalin, and 4 μm 
paraffin sections were stained with hematoxylin and 
eosin (H&E) or by use of Fontana-Masson’s method.

Electron microscopic examinations were conducted 
at one day before UV irradiation and at 1, 2, 3 and 6 
DAI. Small pieces of the skin specimens were fixed in 
2.5% glutaraldehyde and 2.0% paraformaldehyde in 
0.1M PB, postfixed in 1.0% osmium tetroxide in the 
same buffer, and embedded in epoxy resin (Quetol 812, 
Nissin EM Co. Ltd., Tokyo, Japan). Ultrathin sections 
were double-stained with uranyl acetate and lead citrate 
and observed under a JEOL-1200EX electron 
microscope (JEOL Co. Ltd., Tokyo, Japan).

Results

Macroscopic findings

In the irradiated skin, erythematos and edematous 
changes occurred at 1 DAI, and apparent blister 
formation developed in two animals one day later. 
Erythema faded from 3 DAI and almost disappeared at 5 
DAI (Fig. 1). At 6 DAI, the skin recovered to almost 
normal state except for slight desquamation and a few 
scabs. One animal which showed no apparent blister 
formation exhibited more prominent suntan reaction than 
the other two at 5 and 6 DAI.

Histopathological findings

DOPA-positive melanocytes which were 
sporadically seen in the epidermal sheet before UV 
irradiation (Fig. 2a) almost disappeared at 1 and 2 DAI 
(Fig. 2b). At 3 DAI, a small number of melanocytes 
weakly positive for DOPA reappeared, and the number 
of DOPA-positive melanocytes increased over the level 
before UV irradiation at 6 DAI and 2 WAI (Fig. 2c). At 
8 WAI, the number of DOPA-positive melanocytes was 
similar to that before UV irradiation.

On H&E-stained sections, many degenerated 
keratinocytes with vacuolated cytoplasm and pyknotic 
nuclei, so-called «sunburn cells», were observed in the 
epidermis at 1 DAI (Fig. 3b). At the same time, vascular 
dilatation, mild edema and disarrangement of collagen 
bundles were found in the dermis beneath the epidermis. 
At 2 DAI, intercellular edema was prominent in the 
epidermis, and focal epidermal thickening was also 
oberved (Fig. 3c). In addition, in two dogs, blister 
formation with inflammatory cell infiltration (Fig. 3d) 
was evident and the above-mentioned changes in the 
dermis became more striking at 2 and 3 DAI. At 3 DAI, focal 
proliferation of basal cells resulting in formation of 
epidermal ingrowths into the dermis was also 
conspicuous in these two animals (Fig. 3d). In all 
animals, the skin showed almost normal features except 
for slight epidermal thickening at 6 DAI (Fig. 3e), and at 
2 WAI, it exhibited the similar histological features to 
those before UV irradiation (Fig. 3f).

Before UV irradiation, melanin granules were 
distributed in almost the whole length of the epidermis

Fig. 1. Changes in skin color (redness) in hairless dogs after UV 
irradiation measured with a spectrophotometer.
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mainly in the basal layer, and they deposited in the apical portion of each cell forming a «nuclear cap» (Fig. 4a). From 1 to 3 DAI, epidermal cells with melanin pigments were scarce. At 6 DAI, melanin granules were detected only in cells which seemed to be melanocytes in two dogs (Fig. 4b), while marked deposition of melanin granules was observed in one dog which showed no blister formation (Fig. 4c).

Electron microscopic findings

Cytoplasmic vacuolation was conspicuous in the epidermal cells at 1 DAI (Fig. 5a). At 2 and 3 DAI, besides cytoplasmic vacuolation, marked deformation and degeneration of epidermal cells due to severe intercellular edema were observed (Fig. 5b). Moreover, mitosis was also seen especially in the cells which composed epidermal ingrowths (Fig. 5c).

At 1 DAI, melanocytes were characterized by vacuolated cytoplasm, decreased melanosomes, irregular-shaped nuclei and shortened dendrites (Fig. 6a). At 6 DAI, the ultrastructural features of melanocytes returned to normal, and well-developed melanosomes were distributed mainly in the marginal portion and dendrites of melanocytes (Fig. 6b).

Discussion

In this study, dorsal skin reactions exposed to artificial UVA+B irradiation with high energy dose (180kJ/m²) were light and electron microscopically examined in hairless descendants of MHDs.

Macroscopically, prominent erythema developed at 1 DAI, and it began to fade from 3 DAI and almost disappeared at 5 DAI. Such sequence in macroscopic findings was well corresponded with that in histopathological changes in the dermis such as vascular dilatation. In addition, blister formation, which is well known to be seen in humans as one of the sunburn reactions after exposure to high energy dose of solar UV, was found in the present study. However, such severe sunburn reaction was detected neither in the hairless dogs exposed to single low energy dose (40kJ/m²) of artificial UVA+B (Kimura and Doi, 1995), nor in the hairless dogs exposed to solar UV (140 to 150kJ/m²) for 6 consecutive days (Kimura and Doi, 1994a). Generally, severity of UV-induced skin injury is dependent on energy dose and wavelength of UV irradiation. In humans, exposure to multiple minimal erythemal dose (MED) (10 to 15 times MED) can cause a marked sunburn reaction that can lead to severe edema and blistering (Pathak and Fitzpatrick, 1993). In addition, UV of shorter wavelength has more strong biologic effects (e.g. 297 nm radiation is nearly 100 times more erythemogenic than 313 nm radiation) (Kochevar et al., 1993). The artificial UV used in this study contained larger amount of UVB than solar UV (UVA: 5.0 to 6.0 mW/cm² vs. UVB: 0.3 to 0.5 mW/cm² (Taylor et al., 1990)). Thus, severe skin injury might occur in this study.

On the other hand, unlike the previous reports in hairless descendants of MHDs exposed to solar UV (Kimura and Doi, 1994a, 1995), suntan reactions in the present cases were weak (in two dogs) or showed delayed onset (in one dog). This may be related to the severity of the below-mentioned epidermal changes in hairless dogs before and after UV irradiation. DOPA-positive melanocytes (arrowheads), seen before UV irradiation (a), are not detected at 1DAI (b). At 6DAI (c) the number of DOPA-positive melanocytes is more than that before UV irradiation: DOPA reaction. x 320
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the present cases.

Histopathologically, in the epidermis of hairless dogs, sunburn cells, which are said to appear as early as 30 minutes and to be most numerous at 24 hours after UV irradiation in humans (Gilchrest et al., 1981), appeared at 1 DAI. As corresponded with electron microscopic findings, marked intercellular edema, degeneration of epidermal cells and focal epidermal

Fig. 3. Dorsal skin of hairless dogs before (a) and after UV irradiation. Sunburn cells (arrowheads) are seen at 1 DAI (b). At 2 DAI, intercellular edema and focal epidermal thickening are observed (c). At 3 DAI, blister formation and focal proliferation of basal cells are conspicuous (d). At 6 DAI, the skin shows almost normal features except for slight epidermal thickening (e), and, at 2 WAI, it exhibits the similar histological features to those before UV irradiation (f). H&E stain. a-c, e and f, x 150; d, x 60
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Fig. 4. Dorsal skin of hairless dogs before UV irradiation (a) and at 6 DAI (b, c). Before UV irradiation, melanin granules are seen almost in the whole length of the epidermis. However, at 6 DAI, a few cells (probably melanocytes) contain melanin granules in two dogs (b), but marked deposition of melanin granules is observed in one dog without blister formation (c). Fontana-Masson stain. x 300

Fig. 5. Electron micrographs of the dorsal skin epidermis after UV irradiation. Cytoplasmic vacuolation is conspicuous at 1 DAI (a), and marked deformation and degeneration of epidermal cells due to severe intercellular edema were observed at 2 DAI (b). At 3 DAI, mitosis are seen in the cells which composed epidermal ingrowths (c). a, x 3,300; b, x 2,700; c x 2,900
thickening occurred 1 day later. In addition, blister formation was found in two dogs. At the same time, vascular dilatation, edema and disarrangement of collagen bundles were detected in the dermis. These changes however ceased by 6 DAI. Except for their severity, the histopathologic nature and the sequence of skin lesions were fundamentally similar to those in the case of exposure to low energy dose of artificial UVA+B (40kJ/m²) (Kimura and Doi, 1995). The severe forms of dermatological changes seen in this study were closely akin to acute sunburn reactions in humans.

The changes in the number of DOPA-positive melanocytes and the morphologic changes of melanocytes were well correlated. Namely, DOPA-positive melanocytes disappeared from 1 to 3 DAI when melanocytes showed prominent degeneration (Fig. 6a). At 6 DAI, when melanocytes came to show well-developed melanosomes and dendrites (Fig. 6b), the number of DOPA-positive melanocytes increased again. These findings support that suntan reactions were weaker or more delayed in the present case than in the cases exposed to solar UV (Kimura and Doi, 1994a, 1995). In fact, it is considered that epidermal lesions were too severe for the epidermis to develop prominent suntan reactions in this case.

In the present case, melanocytes were damaged by the irradiation of high energy dose of UV. DOPA-positive melanocytes were disappeared from 1 to 3 DAI, and then, the number of DOPA-positive melanocytes were increased over the level before UV irradiation at 6 DAI. The expression of melanocyte-stimulating hormone (MSH) receptor is considered to be involved in UV-induced melanogenesis (Hirobe, 1995). Bologna et al. (1989) showed that UV and topically applied MSH acted synergistically to increase skin darkening in hairless mice and that MSH binding activity was increased by UV irradiation in mouse melanoma cell. Relationship between the activity of melanocytes and expression of MSH receptor remained to be examined in this study. Furthermore, it has also been reported that human melanocytes are activated when they are cultured with proinflammatory mediators, such as arachidonic acid metabolites and histamine (Tomita et al., 1989, 1992). These chemical mediators have possibilities to take part in the activation of melanocytes in the present study.

In hairless descendants of MHDs, epidermal ingrowths into the dermis almost disappeared by 1 year old (Kimura and Doi, 1994b). Interestingly, formation of epidermal ingrowths into the dermis was frequently observed at 3DAI in the present study using 1-year-old hairless dogs. This seems to be due to proliferation of basal cells responsible to epidermal damage. In this connection, mitotic figures were found in such epidermal ingrowths.

In conclusion, hairless descendants of MHDs exposed to high energy dose of artificial UVA+B (180kJ/m²) are useful as a model of severe form of UV-induced dermatological damage in humans.

References


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