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

Y. Ishii[1], T. Kimura[2], S. Itagaki[1] and K. Doi[1]

[1]Department of Biomedical Science, Faculty of Agriculture, The University of Tokyo, Yayoi 1-1-1, Bunkyo-ku and
[2]Research Center, Nihon Nosan Kogyo Co., Ltd., Tsukuba, Ibaraki, Japan

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

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under controlled conditions (temperature: 25±2 ºC; 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 peek in UVA region (365nm) and HP-30M, which has a peek 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/m^2.

In order to evaluate the erythematous 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 (Nagatoisi Co. Ltd., Tokyo, Japan) under local anesthesia with 2% lidocaine at one day before UV irradiation and at 1, 2, 3 and 6 DAI and 2, 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 ºC 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, erythematous 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 observed (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
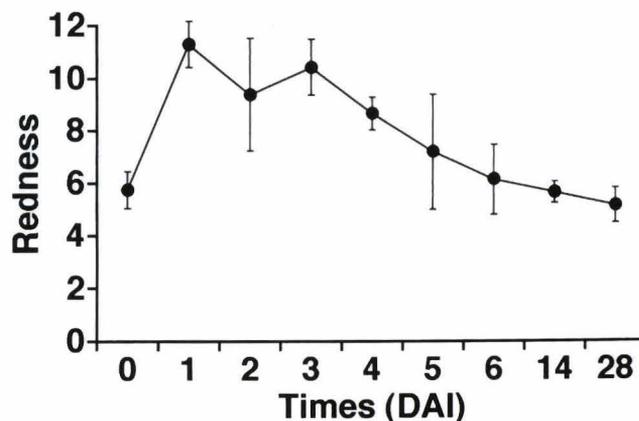

Fig. 1. Changes in skin color (redness) in hairless dogs after UV irradiation measured with a spectrophotometer.

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 ($180kJ/m^2$) 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 ($40kJ/m^2$) of artificial UVA+B (Kimura and Doi, 1995), nor in the hairless dogs exposed to solar UV (140 to $150kJ/m^2$) 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/cm^2 vs. UVB: 0.3 to 0.5 mW/cm^2 (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
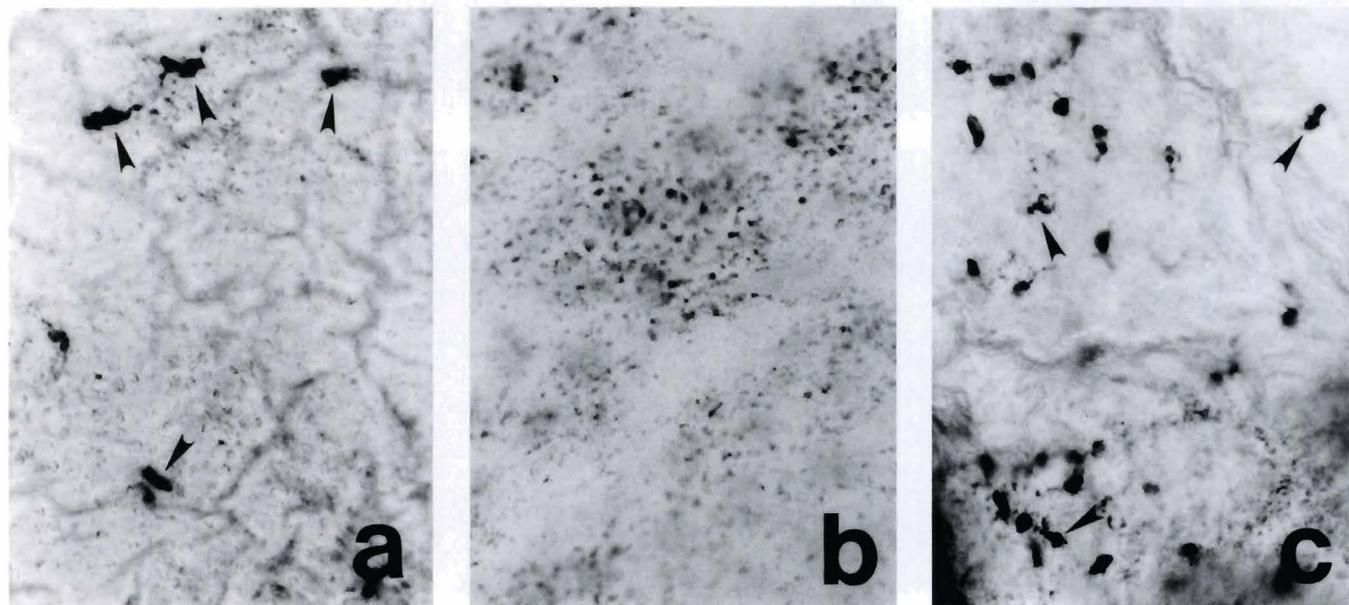

Fig. 2. Epidermal sheet of the dorsal skin of hairless dogs before and after UV irradiation. DOPA-positive melanocytes (arrowheads), seen before UV irradiation **(a)**, are not detected at 1DAI **(b)**. At 6DAI **(c)** the number of DOPA-positive melanocytes is more than that before UV irradiation. DOPA reaction. x 320

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
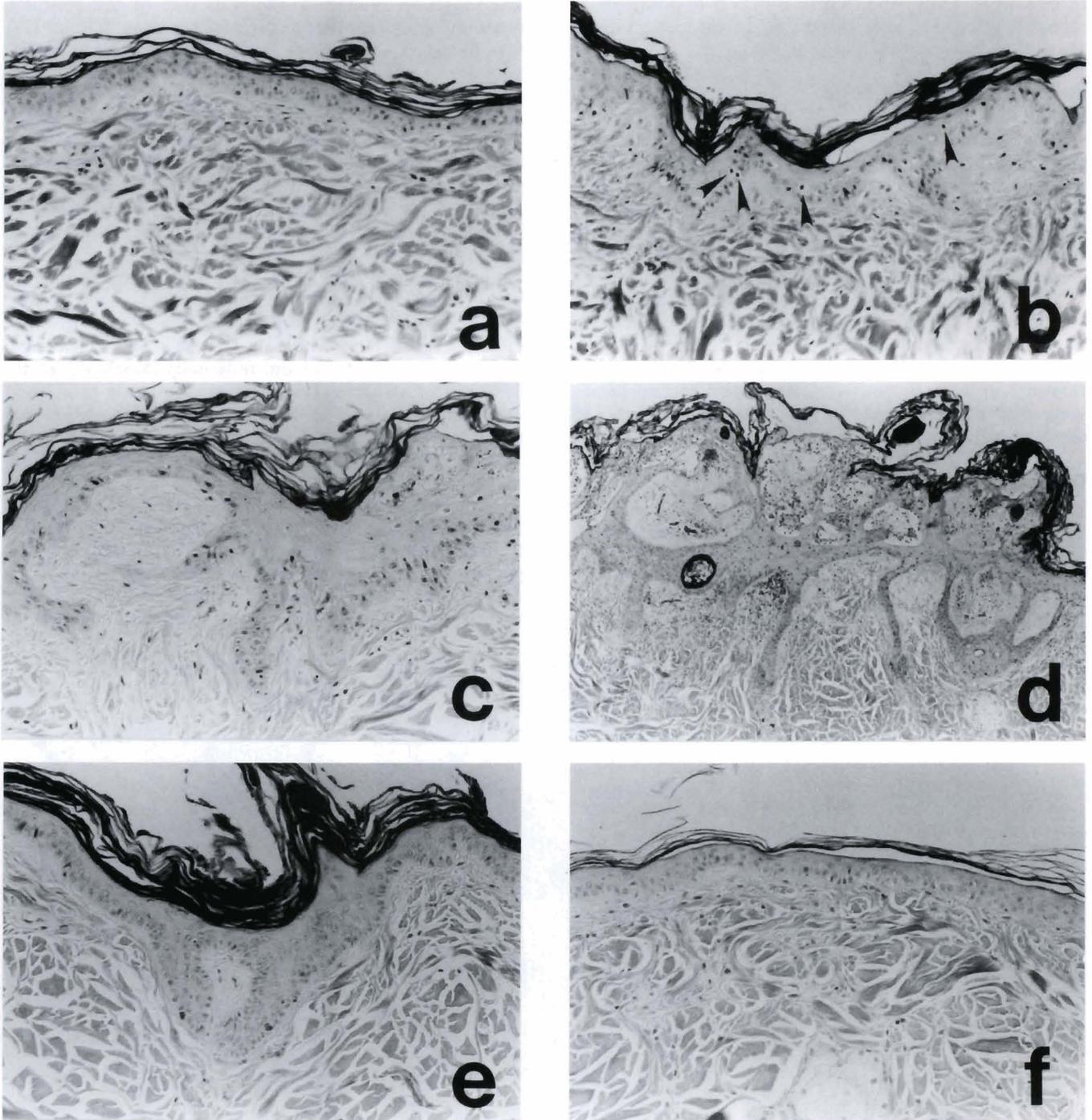

Fig. 3. Dorsal skin of hairless dogs before **(a)** and after UV irradiation. Sunburn cells (arrowheads) are seen at 1 DAI **(b)**. At 2 DAI, intercellular edema and focal epidermal thickening are observed **(c)**. At 3 DAI, blister formation and focal proliferation of basal cells are conspicuous **(d)**. 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
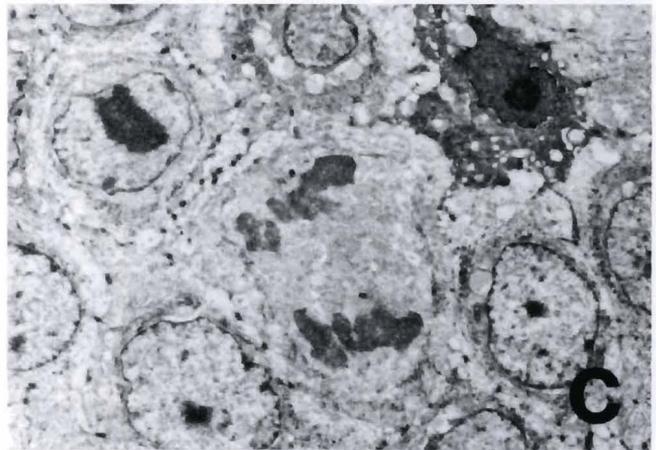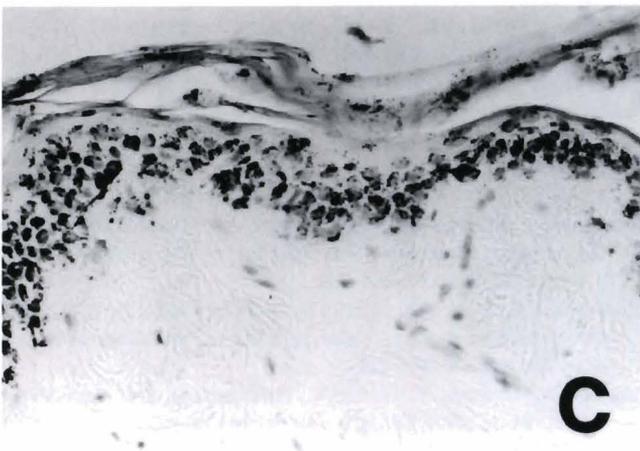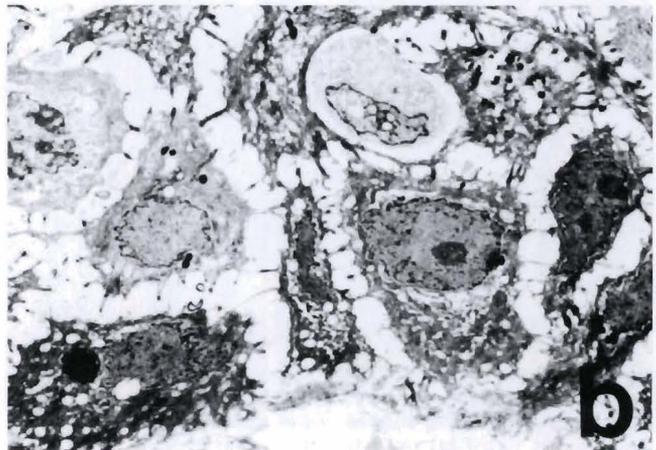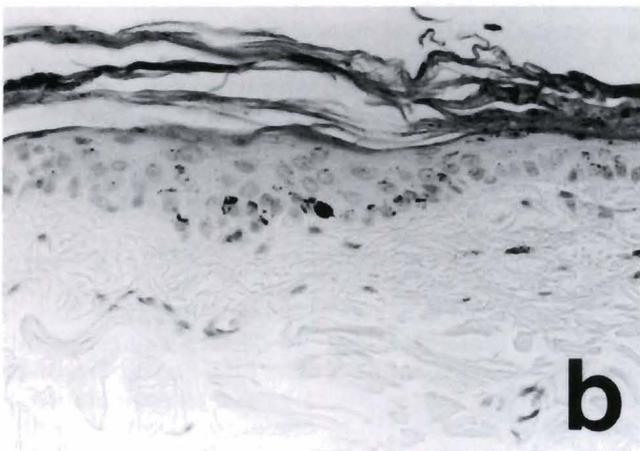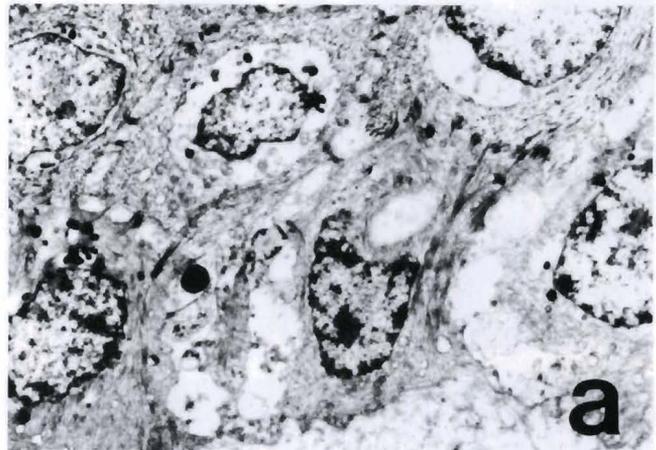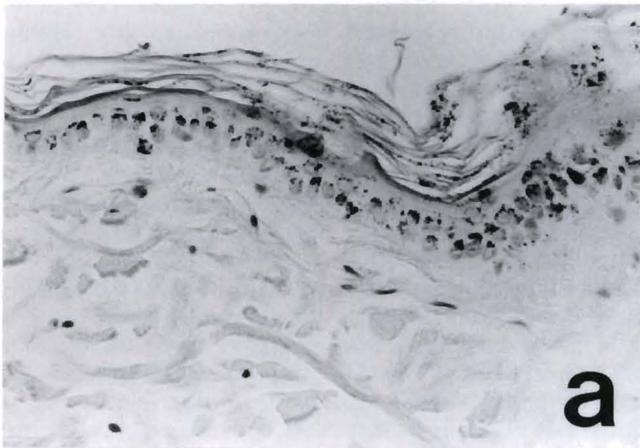
Fig. 4. Dorsal skin of hairless dogs before UV irradiation **(a)** and at 6 DAI **(b, c)**. Before UV irradiation, melanin granules are seen almost in the whole length of the epidermis. However, at 6 DAI, a few cells (probably melanocytes) contain melanin granules in two dogs (b), but marked deposition 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 sever 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
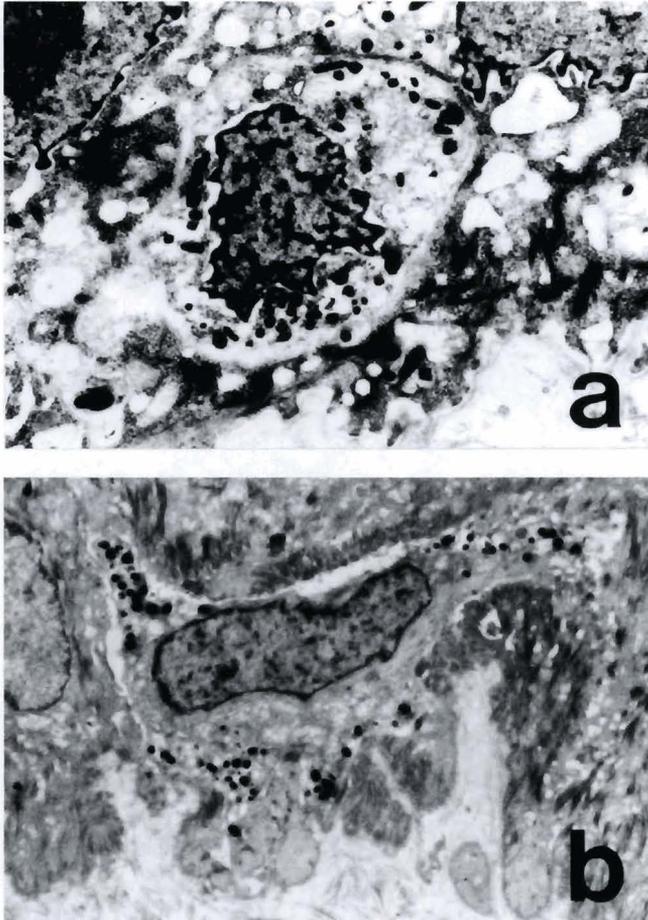
Fig. 6. Electron micrographs of the melanocytes after UV irradiation. A melanocyte, characterized by vacuolated cytoplasm, decreased melanosomes, irregular-shaped nuclei and shortened dendrites is observed at 1 DAI **(a)**. At 6 DAI, the ultrastructural features of melanocytes appear normal, and well-developed melanosomes are distributed mainly in the marginal portion and dendrites of a melanocyte **(b)**. a, x 7,300; b, x 3,500

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 ($40kJ/m^2$) (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). Bolognia 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 ($180kJ/m^2$) are useful as a model of severe form of UV-induced dermatological damage in humans.

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