Histol Histopathol (1998) 13: 29-36

Histology and Histopathology

Effects of indomethacin on sunburn and suntan reactions in hairless descendants of Mexican hairless dogs

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Summary. The inhibitory effects of topical indomethacin (IM)-treatment on sunburn and suntan reactions after ultraviolet (UV)-irradiation were investigated in the dorsal skin of hairless descendants of Mexican hairless dogs. Skin color, plasma prostaglandin E_2 (PGE_2) and histological features were examined. At 1 day after UV-irradiation, the IM-untreated sites showed prominent erythema, while the IM-treated sites exhibited few visible erythematous reactions. From 4 days after UV-irradiation, both the IM-treated and -untreated sites began to develop skin pigmentation. Assessment of skin color changes, using a colorimeter, reflected precisely the color changes in visual sunburn and suntan reactions. Plasma PGE_2 concentration began to increase from 2 hours after UV-irradiation, reached the maximal values at 24 hours and recovered at 96 hours after UV-irradiation. Histologically, at 1 day after UV-irradiation, the IM-untreated sites showed remarkable epidermal degeneration (thickening and sunburn cells) and moderate alteration in the dermis. On the other hand, the IM-treated sites showed only minor histological changes. At 4 days after UV-irradiation, deposition of melanin granules was found in both the IM-treated and -untreated sites. At 7 days after UV-irradiation, pigmentation became more prominent in the *stratum basale*. These results revealed that UV-induced erythematous reactions of hairless dogs were closely related to the action of PGE_2. Visually and histologically, topical IM-treatment had apparent inhibitory effects on erythematous reactions, while this agent showed no protective effects on epidermal pigmentation after UV-irradiation.

Key words: Erythematous reactions, Hairless dogs, Indomethacin, Prostaglandins, Ultraviolet

Introduction

Time of onset and recovery of both sunburn and suntan reactions in hairless descendants of Mexican hairless dogs has been demonstrated to be almost consistent with that in human beings (Kimura and Doi, 1994a). In addition to skin histopathology, ultraviolet (UV)-induced skin reaction in hairless dogs can be quantitatively evaluated by colorimetrical skin color measurement and enumeration of dihydroxyphenylalanin (DOPA)-positive melanocytes (Kimura and Doi, 1994a,b). It has also been shown that the skin reactions exposed to a high energy dose of ultraviolet in hairless dogs resemble those in human beings (Ishii et al., 1997). Therefore, hairless dogs are expected to be useful for the photodermatological investigation.

Prostaglandins (PGs) are the main chemical mediators which induce sunburn (erythematous) reactions in the human skin after UV-irradiation (Mathur and Ghandi, 1972; Black et al., 1978; Gilchrest et al., 1981; Greaves, 1991). Indomethacin (IM) has the property of inhibiting PG synthesis (Vane, 1971). IM is known to be an anti-inflammatory agent which can decrease erythema produced in the human skin with UVB-irradiation when applied topically (Snyder and Eaglestein, 1974a) and intradermally (Snyder and Eaglestein, 1974b). Oral administration of IM also reduced erythema induced with UVB-irradiation (Gruber et al., 1972).

In this study, we evaluated the changes of skin color and histology in order to clarify the inhibitory effects of topical IM-treatment on the occurrence of UV-induced erythema in hairless dogs. In addition, we determined plasma PGE_2 concentrations after UV-irradiation in order to examine the correlation between erythematous reactions and release of PGE_2.

Materials and methods

Dogs

Three 3-year-old male N_2 hairless hybrids (male N_1 hairless hybrids x female Beagles) were used. The dogs were individually housed in steel cages (90x90x90 cm) in an animal room controlled at 25±2 °C and 50±10% relative humidity with 10 to 15 exchanges of 100% fresh air/h and a 12-hr light (7AM to 7PM), 12-hr dark (7PM

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to 7AM), cycle. They were fed a commercial dry dog food (Labo D Standard, Nihon Nosan Kogyo Co., Ltd., Yokohama) and water *ad libitum*.

Test procedures

Three dogs were irradiated with artificial ultraviolet light (UVA+B) (ATTO Co., Ltd., Tokyo, UVB: 290 to 320 nm, UVA: 320 to 400 nm). The irradiation doses of UVA and UVB were 35 kJ/m^2 and 5kJ/m^2, respectively. During these procedures, each dog was placed in a wire cage (85x95x75 cm) with 2 cm mesh. Immediately after UVB-irradiation, the dorsal skin of hairless dogs was treated with indomethacin solution at a rate of approximately 4 μl/cm^2 (Fig. 1). Indomethacin was applied as a 1.0% solution in propylene glycol: ethanol:dimethylacetamide, 19:19:2 (v/v) in 400-μl volumes. Control sites were treated with vehicle alone.

Assessment of skin colors

A spectrophotometer (CR-200, Minolta Co., Ltd., Tokyo, Japan) was used to record skin colors in a 3-dimensional space as follows: Yxy systems (Y= luminance factor, x and y= chromaticity coordinates); L*a*b* system (L*= luminance factor, a*= from redness [+] to greenness [-] and b*= from yellowness [+] to blueness [-]); and LCH systems (L= luminance factor, C= chroma, H= hue) (Kimura and Doi, 1994b). Recording was done at 1 day before beginning of UV-irradiation, and at 1-6 days after UV-irradiation.

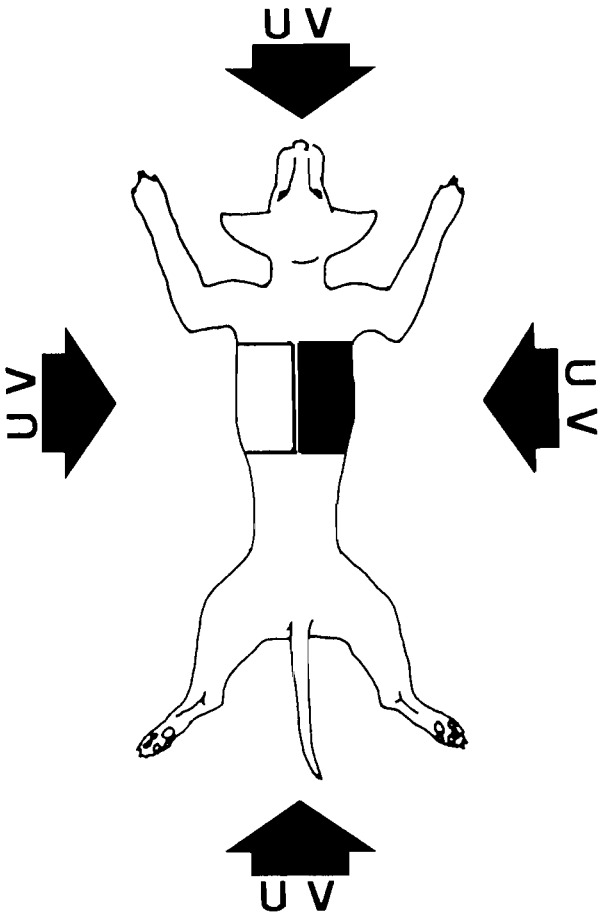

Fig. 1. Diagram of the skin sites examined on the dorsum. black square: IM-treated skin; white square: IM-untreated skin.

PGE_2 assay

Blood samples were drawn from the jugular vein of each animal at one day before and at 2, 24, 48 and 96 hours after UV-irradiation. As an anticoagulant, EDTA-2K-added indomethacin was employed. Blood samples were centrifuged at 1,500g for 10 minutes immediately after the collection of blood, and the plasma was separated and stored at -85 °C until used. Plasma samples were assayed for PGE_2 by use of the radio-immunoassay (the double antibody method).

Histological examination

Tissue specimens were obtained from both the IM-treated and untreated (control) sites of dorsal skin of each animal using a 6A mm biopsy punch (Nagatoishi Co., Ltd., Tokyo) under local anesthesia with 0.5% procaine injected in an annulus surrounding the biopsy punch sites. Specimens were obtained at 1 day before the UV-irradiation, and at 1, 4, and 7 days after irradiation.

Skin specimens were fixed in 10% neutral-buffered formalin, and 4-μm paraffin sections were stained with hematoxylin and eosin (HE) and toluidine blue (TB), and by Fontana-Masson's method (FM).

Statistical analysis

All values were expressed as mean ± standard deviation (SD), and statistical analysis was performed by the Student's t-test.

Results

Macroscopic findings

At 1 day after UV-irradiation, the IM-untreated sites showed prominent erythema, while the IM-treated sites exhibited few visible erythematous reactions. The erythema in the IM-untreated sites peaked at 2 days after UV-irradiation and persisted throughout the next day. Topical IM-treatment had a favorable effect on protecting the skin of hairless dogs from sunburn reaction induced by UV-exposure. At 4 days after UV-irradiation, both the IM-treated and IM-untreated sites began to develop delayed suntan reactions (skin pigmentation). At 7 days after UV-irradiation, skin pigmentation became more prominent.

Skin colors

Assessment of skin color changes by use of a colorimeter correlated well with the color changes in visual erythematous and tanning reactions (Fig. 2). In the Yxy, L*a*b* and LHC systems, the IM-untreated sites had a marked decrease in luminance values (Y, L* and L values) from the first day after UV-irradiation. These values indicated significant differences in the skin pigmentation between the IM-treated and IM-untreated sites ($p<0.05$).

The L*a*b* systems illustrated precisely the changes in erythematous reactions after UV-irradiation. In the IM-untreated sites, the a* values markedly increased at 1 day after UV-irradiation. At 1, 2 and 3 days after UV-irradiation, the a* values in the IM-treated sites were significantly less than those in the IM-untreated sites ($p<0.05$).

The LHC systems also exhibited that IM-treatment was effective in inhibiting development of skin erythematous reactions. In the IM-untreated sites, the C values indicating vivid skin colors increased at 1-3 days after UV-irradiation. Associated with the changes of the C values, the H values (hue angles) decreased. This finding indicated the transition of skin colors into redness. At 1 day after UV-irradiation, there were significant differences in both the C and H values between the IM-treated and IM-untreated sites. These colorimetric values showed that topical IM-treatment favorably inhibited erythema formation in the skin of hairless dogs.

Plasma PGE_2 concentrations

The change of plasma PGE_2 concentrations is shown in Fig. 3. Plasma PGE_2 concentrations were statistically elevated before the appearance of erythema (2 hours after UV-irradiation) ($p<0.01$), and reached their maximum at 24 hours after UV-irradiation. From 24 to 48 hours after UV-irradiation, PGE_2 levels rose approximately 4 to 5 times higher than those before UV-irradiation ($p<0.01$). Subsequently, Plasma PGE_2 values

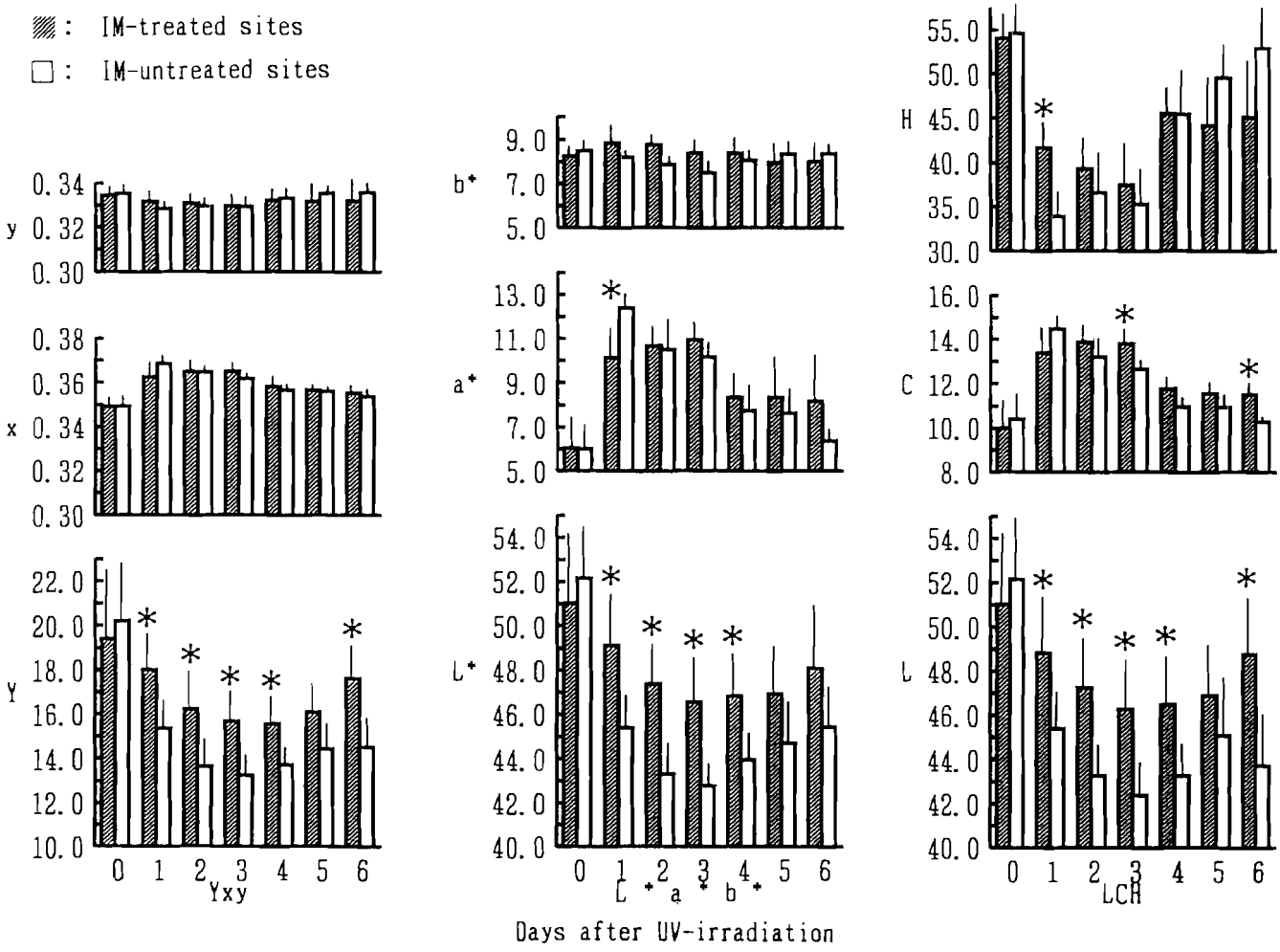

Fig. 2. Changes in dorsal skin colors (mean±SD) of hairless dogs after UV-irradiation. Left: Yxy system; center: L*a*b* system; and right: LCH system. *: $p<0.05$ (significantly different between IM-treated and IM-untreated sites).

declined and returned to the baseline at 96 hours after UV-irradiation.

Histological changes

At 1 day after UV-irradiation, the IM-untrated sites showed prominent sunburn reactions. The epidermis became thickened with cellular and/or intracellular edema (Fig. 4). In some portions, a few epidermal cells with pyknotic nucleus and eosinophilic cytoplasm known as sunburn cells were seen mainly in the *stratum basale* (Fig. 5). In the dermis beneath the degenerative epidermis, moderate alteration in connective tissues was also found. On the other hand, the IM-treated sites showed considerable alleviation of erythematous changes in the epidermis and dermis (Fig. 6).

At 4 days after UV-irradiation, in the IM-untreated sites, the epidermis still showed thickening, and deposition of melanin granules increased especially in the *stratum basale* (Fig. 7). Deposition of melanin granules was also found in the IM-treated sites (Fig. 8).

At 7 days after UV-irradiation, both the IM-treated and IM-untrated sites had more prominent melanin pigmentation mainly in the *stratum basale* (Figs. 9, 10), and topical IM-treatment did not protect the skin of hairless dogs from delayed suntan reaction after UV-irradiation. In the IM-untreated sites, the skin recovered from UV-induced inflammatory reactions.

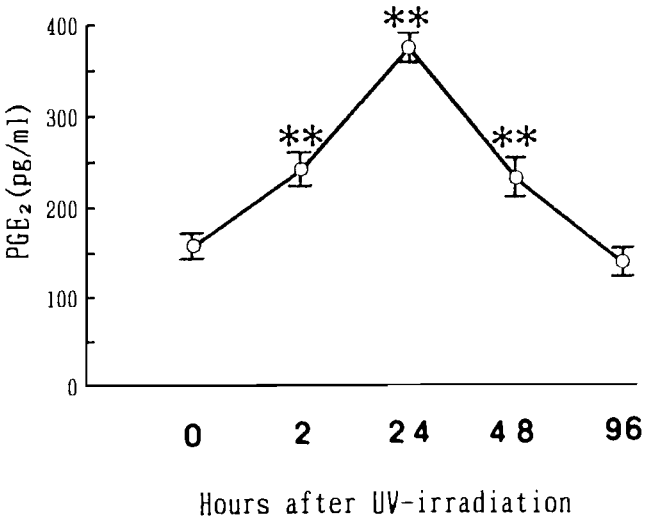

Fig. 3. Changes in plasma PGE_2 concentrations (mean±SD) after UV-irradiation. **: $p<0.01$ (significantly different from pre-irradiation).

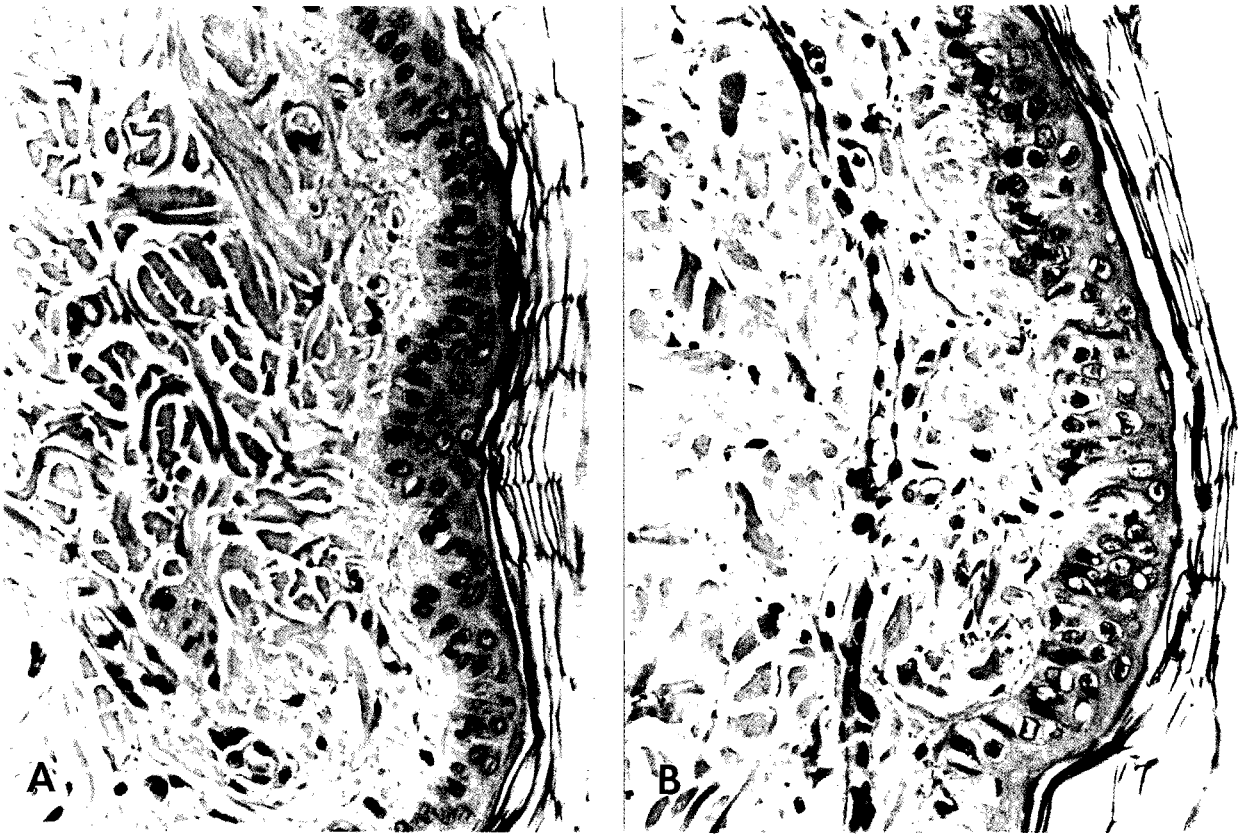

Fig. 4. Dorsal skin of a hairless dog. **A.** before UV-irradiation. **B.** IM-untreated sites at 1 day after UV-irradiation. Epidermal thickening and degeneration are seen. HE stain, x 350

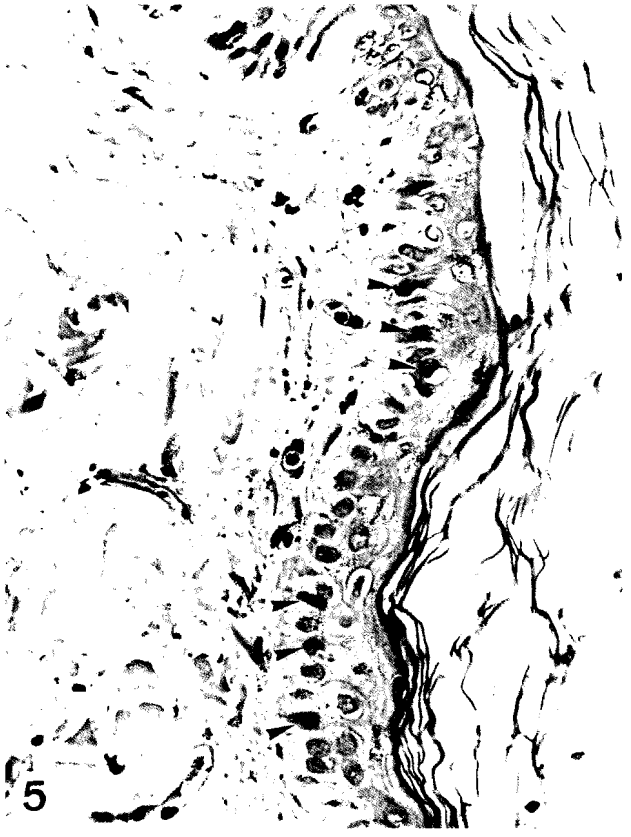

Fig. 5. Dorsal skin untreated with IM at 1 day after UV-irradiation. Sunburn cells (arrowheads). HE stain, x 350

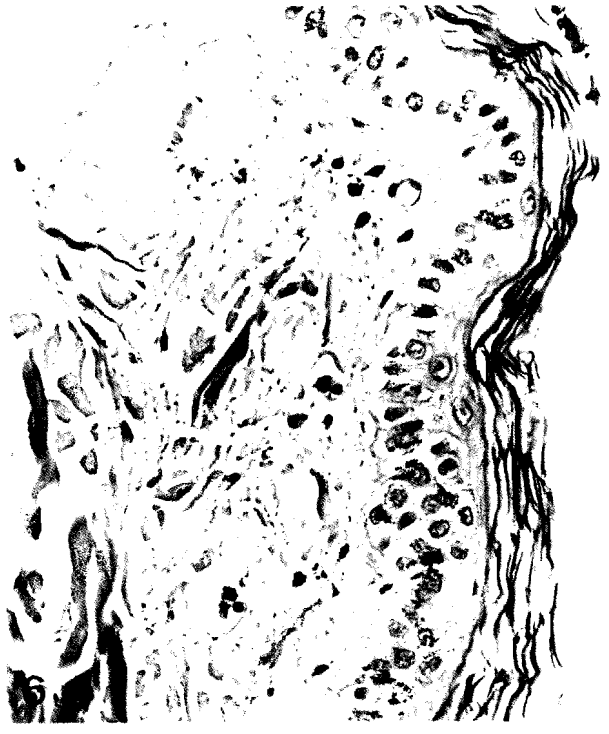

Fig. 6. Dorsal skin treated with IM at 1 day after UV-irradiation. No prominent changes in the epidermis and dermis are seen. HE stain, x 350

Discussion

After UV-irradiation, time onset of the visible changes of skin colors in hairless dogs including sunburn and suntan reactions seems to be consistent with that in human beings (Satoh, 1991). The use of a colorimeter allowed accurate numerical assessment of the changes in visual skin colors. Recently, the numerical evaluation method using a colorimeter contributes greatly to estimation of erythema and/or pigmentation in other diseases such as psoriasis (Takiwaki and Serup, 1994) and nevus (Aubin et al., 1991), and in skin tests (Pierard and Pierard, 1993) and inflammatory reactions (Nose and Tsurumi, 1993). Seitz and Whitmore (1988) and Chardon et al. (1991) reported that the a* values in the L*a*b* systems most closely approximated the erythema of human skin. The present study in hairless dogs indicated that these indices correlated well with increasing erythematous reactions of the skin after UV-irradiation. In the LCH systems, the C and H values represented properly the degree of erythematous reactions of the UV-irradiation skin of hairless dogs.

Visible suntan reactions observed in the skin of hairless dogs were well reflected in the luminance values, as reported in our previous studies (Kimura and

Fig. 7. Dorsal skin untreated with IM at 4 days after UV-irradiation. The epidermal thickening with development of pigmentation of melanin granules in the *stratum basale*. FM stain, x 350

Doi, 1994a,b). Maeda et al. (1996) reported that the values of index Y might relate mainly to content of melanin pigment of the *stratum basale*. Our results in UV-exposed hairless dogs were in close agreement with this finding in human beings.

Morison et al. (1977) reported that blanching of UVB-induced delayed erythema was seen at the IM-treated sites in human skin for 48 hours after UVB-irradiation. Vane (1971) reported that IM was approximately 45 times more effective in inhibiting PG biosynthesis than aspirin. In hairless dogs, the IM-treated sites exhibited the prominent inhibitory efficacy against erythematous reactions after UV-irradiation.

The changes of plasma PGE_2 concentrations corresponded to the manifestation and/or resolution of erythematous reactions of the UV-irradiated skin in hairless dogs. In the human sunburn reactions, Gilchrest et al. (1981) reported that PGE_2 levels were statistically elevated before the manifestation of erythema and reached approximately 150% of the control values at 24 hours. Our UV-exposure study in hairless dogs accorded with these results in human beings. Gilchrest et al. (1981) demonstrated that histamine levels rose approximately fourfold above control values immediately after the onset of erythema and returned to baseline within 24 hours. Logan and Wilhem (1966) found that administration of an antihistamine drug prevented UV-induced early vasopermeability only in guinea pigs, whose mast cell granules contained predominantly histamine. It seems that there are species differences in chemical mediator of UVB-induced erythema. The present study suggests the following histamine and PGE_2 may mediate synergistically the early phase of UV-induced sunburn reactions, and then PGE_2 may play at least some roles in the later phase; such as visible erythematous reactions and histological changes in the skin of human beings and hairless dogs.

Histological examinations revealed that topical IM-treatment had apparent inhibitory effects on erythematous reactions after UV-irradiation. At 1, 4 and 7 days after UV-irradiation, dermatological changes in the IM-untreated sites of hairless dogs were similar to those reported in our previous study (Kimura and Doi, 1995). UV-induced changes such as erythematous reactions and melanin pigmentation observed in hairless dogs were in close accord with those in human beings. These histological changes were closely connected with alterations in skin color and the levels of plasma PGE_2.

The IM-treated sites showed histologically few erythematous reactions. These histological findings were

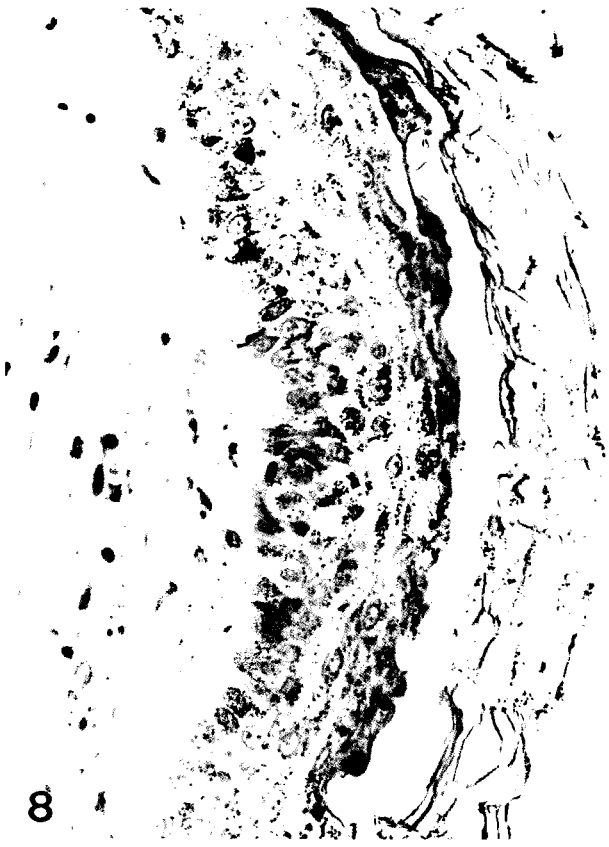

Fig. 8. Dorsal skin treated with IM at 4 days after UV-irradiation. Pigmentation is found in the epidermis. FM stain, x 350

Fig. 9. Dorsal skin untreated with IM at 7 days after UV-irradiation. Pigmentation is seen. FM stain, x 350

Fig. 10. Dorsal skin treated with IM at 7 days after UV-irradiation. Pigmentation is seen. FM stain, x 350

parallel to the changes in both skin color and colorimetric parameters. Snyder and Eaglestein (1974b) and Gruber et al. (1972) reported that nonsteroidal anti-inflammatory agents such as aspirin and IM which were given topically and systematically could decrease the erythema produced in human skin by UVB-irradiation.

In our histological results, topical IM-treatment reduced adequately erythematous reactions, while this chemical agent had no protective effects on epidermal pigmentation after UV-irradiation. Morison et al. (1977) reported that the UVB-irradiated sites that had blanched with topical IM-treatment represented the same degree of pigmentation as comparable sites treated with vehicle alone or left untreated. It is likely that PGE_2 had no association with melanogenesis in the skin of hairless dogs and human beings.

In conclusion, it was clarified that UVB+A-induced erythematous reactions of hairless dogs were closely related to the action of PGE_2. In addition, clinical observations, colorimetric assessment and histological examinations revealed that topical IM-treatment inhibited sunburn reactions in the skin of hairless dogs after UVB+A irradiation. These characteristic similarities of the skin between hairless dogs and human beings make this laboratory animal the most appropriate model for photodermatological investigations.

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Accepted April 6, 1997