Histol Histopathol (2002) 17: 683-690

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Dorsal skin responses to subchronic ultraviolet B (UVB)-irradiation in Wistar-derived hypotrichotic WBN/ILA-Ht rats

V. Malcotti[1], A. Yasoshima[1], K. Imaoka[2], H. Nakayama[1] and K. Doi[1]

[1]Department of Veterinary Pathology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Yayoi, Bunkyo-ku, Tokyo and [2]Department of Microbiology, National Institute of Public Health, Shirokanedai, Minato-ku, Tokyo, Japan

Summary. Dorsal skin responses to a subchronic UVB-irradiation (10kJ/m^2/rat /day), were examined in Wistar-derived hypotrichotic WBN/ILA-*Ht* rats for up to 3 months. Hyperplasia of epidermal cells and hair follicle epithelial cells as well as parakeratosis developed at 1 month and progressed thereafter, resulting in a prominent epidermis thickening and formation of epidermal ingrowths projecting into the dermis. At the same time, the percentage of proliferating cell nuclear antigen (PCNA)-positive epidermal cells significantly increased after 1 month. In some portions of the hyperplastic epidermis, especially of the epidermal ingrowths, keratinocytes were somewhat pleomorphic and migrated into the dermis. In the upper dermis, edema with capillary congestion, mast cell infiltration and fibroblast proliferation developed at 1 month, and the intensity of edema and the number of dermal mast cells was most prominent at 3 months. Edema spread to the epidermis, resulting in intercellular edema and subsequent dissociation of epidermal cells. Degeneration of collagen fibers was also detected in the upper dermis, especially beneath the epidermis. In addition, although not significant because of a large individual difference, the serum IgE concentration, showed a tendency to increase after 2 months. The present study clarified the characteristics of the dorsal skin responses to a subchronic UVB-irradiation in rats.

Key words: Skin responses, Subchronic UVB-irradiation, Histology, Serum IgE, WBN/ILA-*Ht* rat

Introduction

Solar ultraviolet (UV) radiation, especially UVB (290-320 nm), is very important for human beings, because it has positive effects on their psychological well-being and promotes the production of vitamin D_3 in vivo. These effects, however, are offset by a number of reverse effects (Ambach and Blumthaler, 1993). Up to the present time, many studies have been performed on the skin exposed to a single acute dose of UV radiation (Anderson and Parrish, 1981; Zamansky and Chou, 1987; Benjamin et al., 1991; Brash et al., 1991; Devary et al., 1993; Nishimura et al., 1999; Kuroki et al., 2001; Malcotti et al., 2001a,b). On the other hand, there are only a small number of reports of the detailed skin responses to a long-term UV-irradiation, although it is said that a chronic exposure to sunlight leads to solar dermatoses and premature aging of skin (Zamansky and Chou, 1987; Benjamin et al., 1991; Rice and Cohen, 1996), and the accumulation of UV photodamage in the DNA of skin cells resulting from a chronic exposure to sunlight is considered to be responsible for the initiation of skin cancer (Jones, 1997; Mitchell et al., 2001). The relationship between chronic exposure to UVB and photocarcinogenesis is however not fully understood (Mitchell et al., 2001). To clarify this point through comparative studies using various animal models is very important.

In this study, as the first step, we examined the dorsal skin responses to a subchronic UVB-irradiation in WBN/ILA-*Ht* rats. WBN/ILA-*Ht* rats have an autosomal dominant gene (*Ht*) responsible for their characteristic of hypotrichosis (Nishimura and Ishikawa, 1987) and are considered to be very useful in the field of dermatotoxicology (Iwamoto et al., 1997; Kuroki et al., 1998; Albarenque et al., 1999).

Materials and methods

Animals

Twenty-four 7-week-old male WBN/ILA-*Ht* rats (Saitama Experimental Animal Supply Co., Saitama, Japan) were used. They were individually kept in isolator cages in an animal room under controlled conditions (temperature, 23±2 °C; relative humidity, 55±5%; ventilation, 14-16 times/hr; lighting, 14-hr

Offprint requests to: Dr. Valeria Malcotti, Department of Veterinary Pathology, Graduate School of Agriculture and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan. Fax: +81-3-5841-8185. e-mail: aa87181@mail.ecc.u-tokyo.ac.jp

light/10-hr dark cycle), and were fed commercial pellets (MF, Oriental Yeast Co., Tokyo, Japan) and tap water ad libitum. The Laboratory Animal Use and Care Committee of the Graduate School of Agricultural and Life Sciences, The University of Tokyo, approved the study.

Treatment

Fifteen animals were daily exposed to an artificial UVB light (HP-15M, discharge tube type T-15M, wavelength: 312 nm) (ATTO Co., Tokyo) at 10 cm below the light source for 70 min a day (irradiation dose 10kJ/m^2) except on Sunday for up to 3 months. The dose and irradiation procedures were decided based on the results of our previous studies (Kuroki et al., 2001; Malcotti et al., 2001a,b). Namely, clear erythema, intradermal inflammation and epidermal degeneration were induced and almost subsided 24 hours after a single irradiation when the present irradiation procedure and dose were employed. The nine untreated animals served as controls.

Histology

Five animals of the UVB-irradiated group were killed by exsanguination under ether anesthesia at 1, 2 and 3 months after the beginning of the experiment, respectively. Necropsy was performed at 24 hours after the last irradiation. The animals of the control group were killed in the same way. Blood samples obtained from each animal at necropsy were subjected for the measurement of serum IgG and IgE concentrations.

Dorsal skin and lymphoid tissues (thymus, spleen and cervical and mesenteric lymph nodes) were fixed in 10% neutral-buffered formalin, and paraffin sections (4 μm) were stained with hematoxilin and eosin (HE) and toluidine blue (TB). Some sections of the skin were stained by Masson's trichrome (TC) and Elastica van Gieson's stainings for the examination of connective tissue components. The skin of ears and neck where scratch and scar were observed was excluded from histological examinations.

In addition, small pieces of the dorsal skin obtained from each animal were fixed in 2.5% glutaraldehyde in 0.1M phosphate buffer (pH 7.4), postfixed in 1% osmium tetroxide in the same buffer and embedded in Epok 812 (Ohken Co., Ltd., Tokyo). Then, semi-thin sections (1 μm) were stained with TB for histological examinations.

The number of mast cells in the dermis was counted on the TB-stained paraffin section (average number/mm skin section/animal) under light microscope (x200) in 3 fields, and a mean value was calculated for each animal. The data were expressed as mean ± standard deviation (SD) of 3 (control group) or 5 animals (UVB-treated group) at each point of examination.

Immunohistochemistry for proliferating cell nuclear antigen (PCNA)

To evaluate the proliferating activity of epidermal cells, immunohistochemical staining for PCNA was performed on the above-mentioned paraffin sections by the avidin-biotin peroxidase complex method using an ABC kit (Vector Laboratories, USA). Mouse anti-rat PCNA antibody (clone PC10; Novocastra, Newcastle, UK) was used as the primary antibody. The sections were visualized by diaminobenzidine (DAB) reaction and then counterstained with methylgreen.

PCNA-positive cells in the epidermis were counted on the immmunohistochemically-stained section in the same way as that used for the number of mast cells.

Serum IgG and IgE concentrations

Serum IgG and IgE concentrations were measured by sandwich ELISA method using biotinylated and non-biotinylated anti-rat IgG and IgE mouse monoclonal antibodies (MAbs) (Zymed Laboratories, San Francisco, USA). In brief, microplate wells (Dynatech, Chantilly, VA) were coated with an anti-rat IgG or IgE MAb at 37 °C for 3 hrs and then incubated with phosphate-buffered saline (PBS) containing 1% bovine serum (Sigma Chemicals Co., St. Louis, MO, USA) for 1 hr at room temperature. After washing with PBS containing 0.05% Tween 20 (PBST) (Nakalai Tesque, Kyoto, Japan), serially diluted serum samples were placed in microplate wells and incubated overnight at 4 °C. After washing with PBST, biotinylated anti-rat IgG or IgE MAb was added to each well and incubated for 1 hr at room temperature. After washing, the wells were incubated with horseradish peroxidase-conjugated streptavidin (Sigma) (for IgG) or D-galactosidase-conjugated streptavidin (Gibco BRL, Tokyo) (for IgE) for 1 hr at room temperature. After the final washing, the wells were incubated with *o*-phenylendiamine dihydrochloride (Sigma) (for IgG) for 1 hr at room temperature or with 4-methylumbelliferyl-ß-D-galactoside (Sigma) (for IgE) for 2 hrs at 37 °C.

The absorbance was measured by a microplate ELISA reader (BIO-RAD, California, USA) for IgG while the fluorescence intensity was measured using a microplate fluorescence reader (Fluoroscan Fow Laboratories, Costa Mesa, CA, USA) for IgE. Total IgE concentrations were determined from a standard curve obtained from a rat standard IgE (Zymed). One sample each obtained from the UVB-treated group at 1 month and another obtained at 3 months were excluded from the measurement of serum Ig levels because of marked hemolysis.

Statistical analysis

Statistical analysis was done on the data of the percentage of PCNA-positive cells, the number of mast

cells and the concentrations of serum IgG and IgE using Student's t-test or Mann-Whitney's U test.

Results

Gross findings of the dorsal skin

Marked erythema developed within the first week of UVB-irradiation and it gradually subsided thereafter. The dorsal skin became rough and thickened, and desquamation of abnormally thickened corneum also increased with the day of irradiation. In some animals, scratching and subsequent scar formation were observed at the skin of ears and neck at 20 days of UVB-irradiation, and their incidence increased thereafter.

Histological findings of the dorsal skin

At 1 month of UVB-irradiation, as compared with the control skin (Fig. 1), hyperplasia of epidermal cells and hair follicle epithelial cells as well as parakeratosis were observed (Fig. 2). These cells were generally hypertrophic, had enlarged nucleoli, and were sometimes under mitotic process. They were dissociated from each other due to intercellular edema. The epidermis-dermis border became irregular in some portions. In the upper dermis, edema with capillary congestion and dilatation, proliferation of fibroblasts, and infiltration of mast cells were observed (Fig. 2).

At 2 and 3 months of UVB-irradiation, hypertrophy and hyperplasia of epidermal cells and hair follicle epithelial cells as well as parakeratosis progressed, and epidermal ingrowths were frequently found projecting into the dermis (Fig. 3). In the epidermis including epidermal ingrowths, keratinocytes were somewhat pleomorphic and showed prominent nucleoli or mitotic figures (Fig. 4). In some portions, the epidermis-dermis border became indistinct, and proliferated keratinocytes migrated into the dermis (Fig. 4). In the dermis, the similar changes to those observed at 1 month progressed, and edema and mast cell infiltration were prominent especially at 3 months (Fig. 5). As shown in Fig. 6, the number of mast cells increased with the day of UVB-irradiation.

As to the connective tissue components, collagen fibers made up thick bundles throughout the dermis in the control skin (Fig. 7a). On the other hand, in the UVB-irradiated skin, collagen bundles in the upper dermis, especially beneath the epidermis, were broken

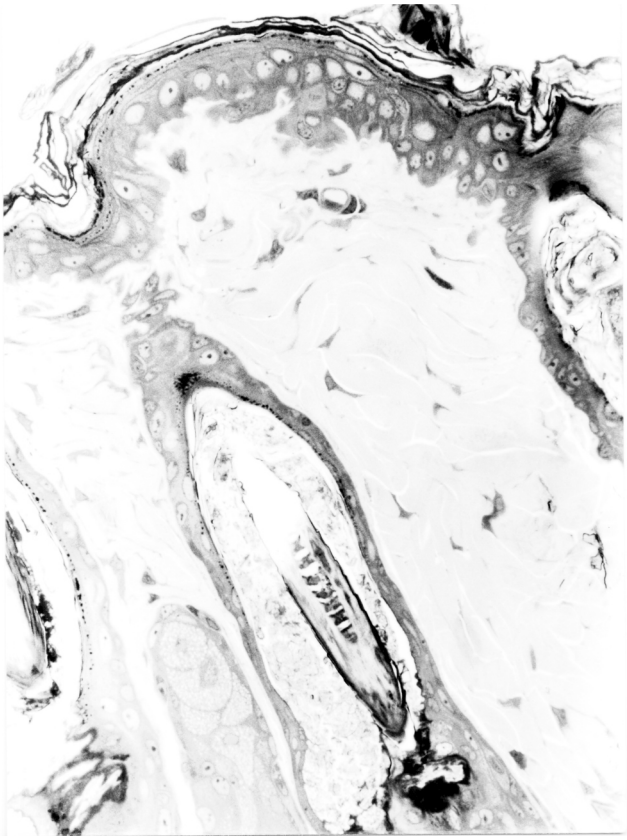

Fig. 1. Dorsal skin of a control rat. Semi-thin section stained with TB. x 350

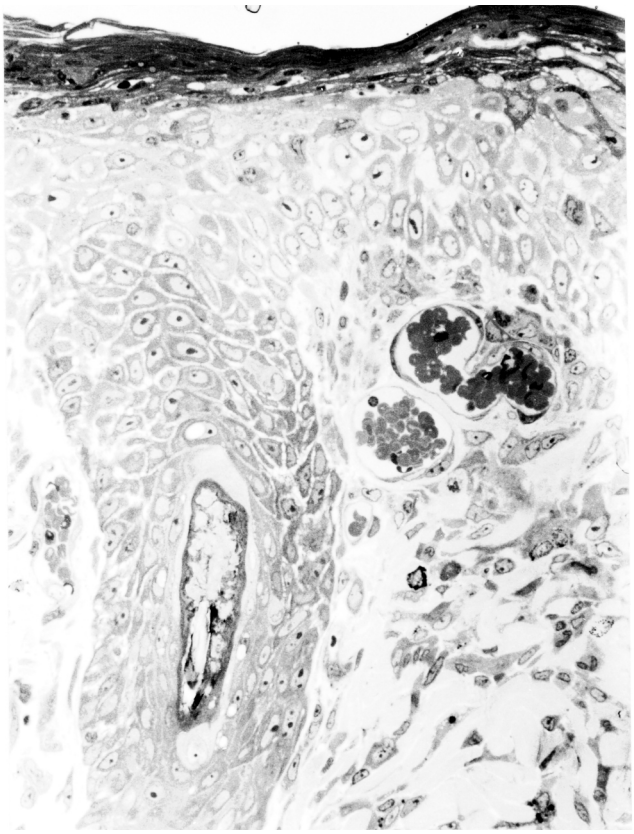

Fig. 2. Dorsal skin of a UVB-irradiated rat at 1 month. Parakeratosis, epidermal hyperplasia with intercellular edema, dermal edema with capillary congestion and fibroblast proliferation are seen. Semi-thin section stained with TB. x 350

down into small fragments (Fig. 7b) or dissociated into fine fibrils (Fig. 7c). These changes progressed with the day of UVB-irradiation. At the same time, an apparent increase in the amount of collagen was observed in the middle and deeper dermis, resulting in the increase of dermal thickness. There were no changes observed in elastic fibers.

Histological findings of lymphoid tissues

There were neither degenerative nor suppressive changes observed in the thymus, spleen and cervical and mesenteric lymph nodes even in animals killed at 3 months of UVB-irradiation.

PCNA-positive cells

In the control skin, PCNA-positive cells were found in the basal layer of the epidermis (Fig. 8a), and the number of PCNA-positive cells was about 20 (Fig. 9). In the UVB-irradiated skin, the number of PCNA-positive cells was about 50 at 1 month, markedly increased thereafter, and reached a value of about 90 at 3 months (Fig. 9). PCNA-positive cells were observed in the basal layer of the epidermis, including epidermal ingrowths, and in the outer layer of the hair follicle epithelia (Fig. 8b). Immunoreactivity for PCNA was also observed within a small mass of keratinocytes forming small-sized epidermal ingrowths (Fig. 8b) or showing a focal epidermal hyperplasia (Fig 8c).

Serum IgG and IgE concentrations

Serum IgG concentrations (7-10 mg/ml) were not different between the control and the UVB-irradiated animals at any point of examination. On the other hand, although not significant because of a large individual difference, serum IgE concentrations showed a tendency to increase after 2 months of UVB-irradiation as compared with the control animals (Fig. 10).

Discussion

In the present study, the dorsal skin responses to a subchronic UVB-irradiation were examined in hypotrichoticWBN/ILA-*Ht* rats.

In the dorsal skin of WBN/ILA-*Ht* rats exposed to an acute UVB-irradiation, an appearance of so-called

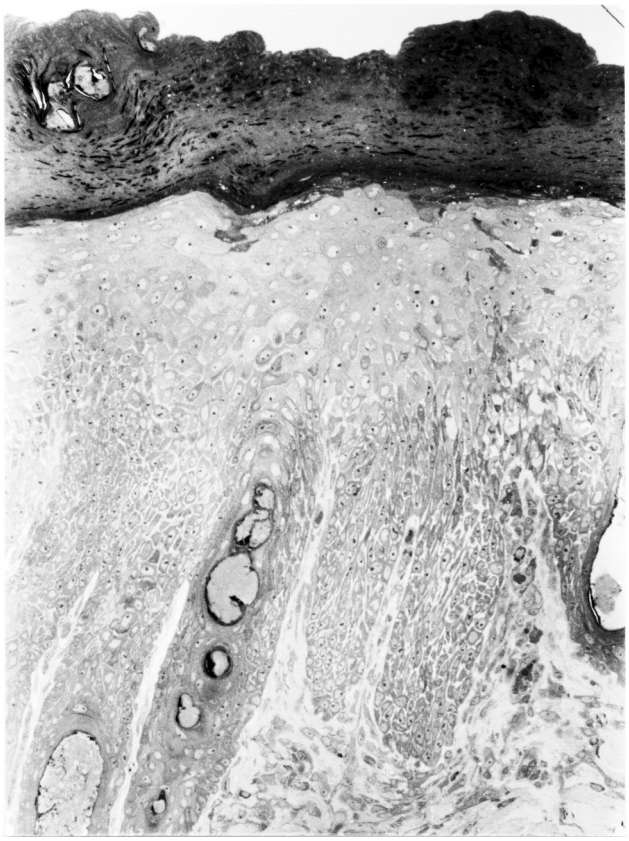

Fig. 3. Dorsal skin of a UVB-irradiated rat at 2 months. Prominent parakeratosis, epidermal cell hypertrophy and hyperplasia together with epidermal ingrowths projecting into the dermis are seen. Semi-thin section stained with TB, x 175

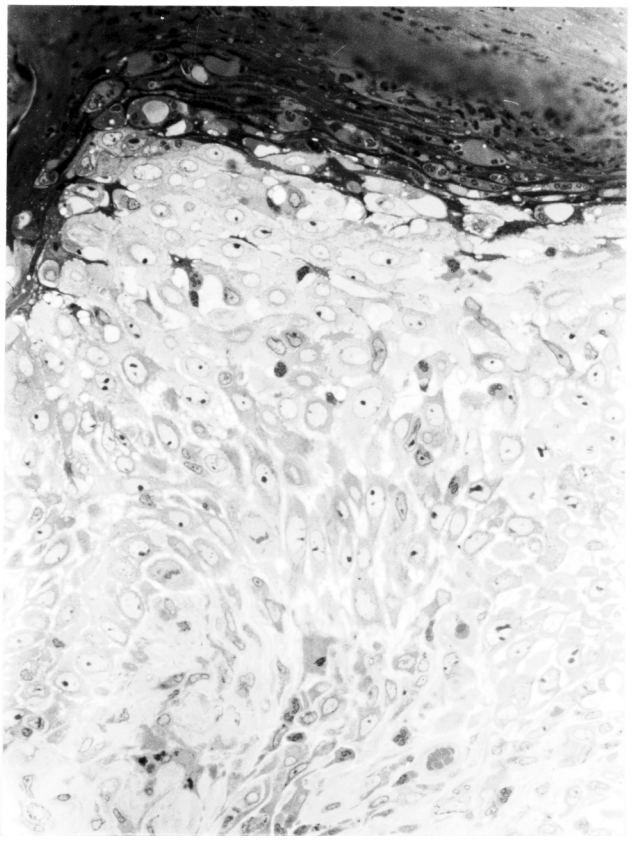

Fig. 4. Dorsal skin of a UVB-irradiated rat at 3 months. Epidermal cells are somewhat pleomorphic with prominent nucleoli. They are frequently under mitotic process. Some of them are migrating into the dermis. Semi-thin section stained with TB, x 350

sunburn cells and subsequent keratinocyte hyperplasia in the epidermis and inflammatory cell infiltration and edema with vascular dilatation in the dermis were characteristic histological features (Kuroki et al., 2001; Malcotti et al., 2001b). Sunburn cells showed

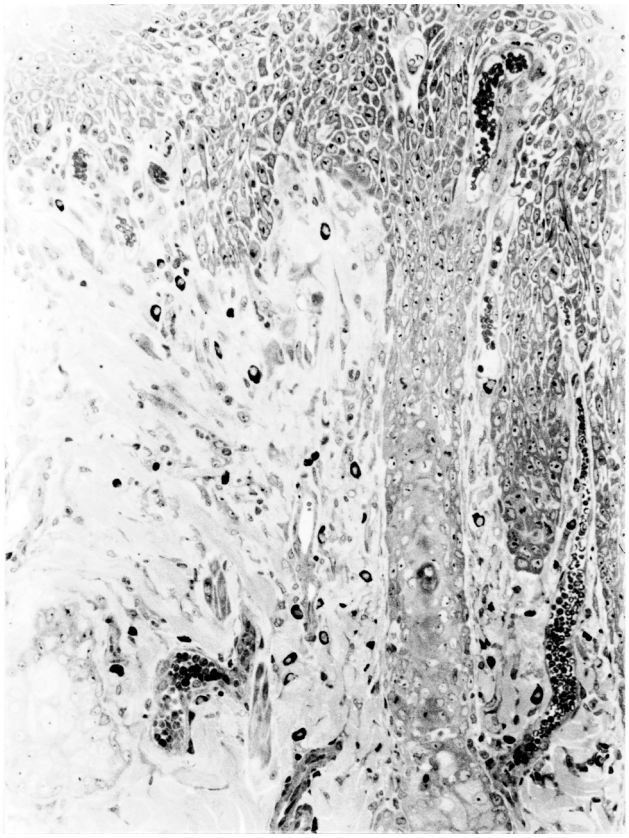

Fig. 5. Dorsal skin of a UVB-irradiated rat at 3 months. Many mast cells are seen in the edematous dermis with capillary congestion. Semi-thin section stained with TB, x 175

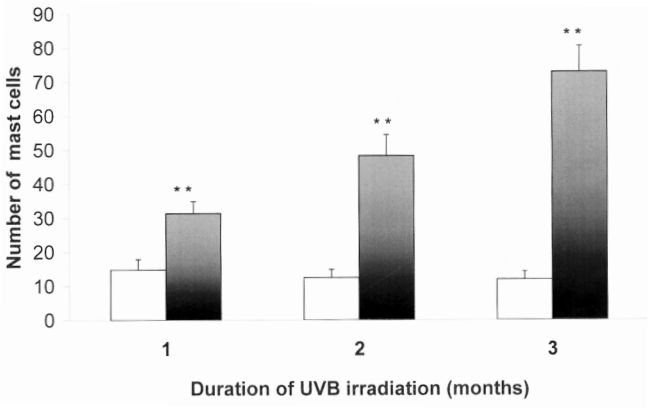

Fig. 6. Changes in the number of mast cells in the dorsal skin dermis of UVB-irradiated rats. Data are shown as mean ± SD of 3 (control group, white square) or 5 rats (UVB-irradiated group, black square). **: $p<0.01$, significantly different from control.

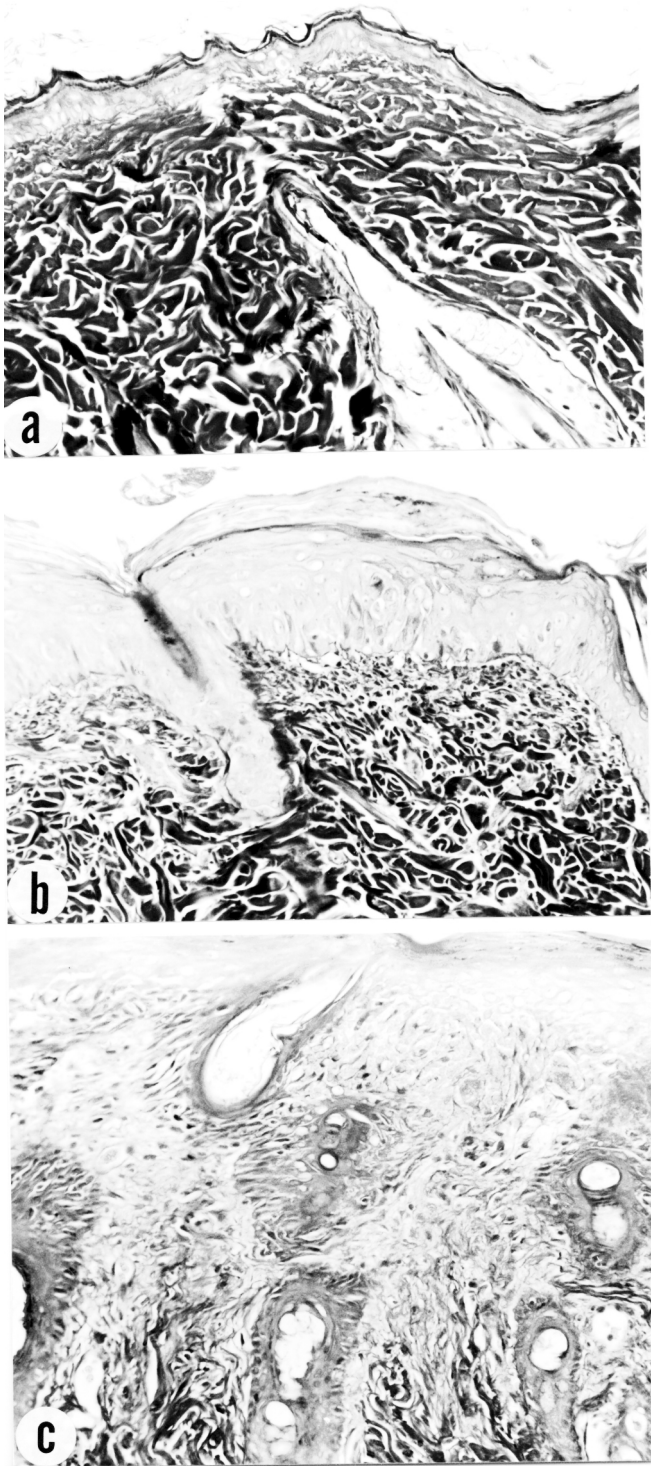

Fig. 7. Dorsal skin of a control rat **(a)**, and UVB-irradiated rats at 2 months **(b)** and 3 months **(c)**. Collagen bundles are broken down into small fragments beneath the epidermis **(b)**, and they are disintegrated into fine fibrils in the upper dermis **(c)**. TC, x 175

ultrastructural characteristics for apoptosis (Kuroki et al., 2001; Malcotti et al., 2001a), and they are considered as a cell population of keratinocytes undergoing apoptosis (Young, 1987; Haake and Polakowska, 1993; Kane and Maytin, 1995). The induction of apoptosis has been considered to play a physiologically important role in eliminating DNA-damaged cells from the skin in the case of acute UVB-irradiation, and it is reported that UVB also activates a proliferative pathway of keratinocytes to replace apoptotic cells (Ouhtit et al., 2000). In the present subchronic UVB-irradiation study, sunburn cells were hardly found throughout the experimental period while epidermal hyperplasia was prominent.

In the present subchronic UVB-irradiation study, hyperplasia of epidermal cells and hair follicle epithelial cells was obvious, and it progressed with the day of UVB-irradiation. As a result, the epidermis itself became prominently thickened. In addition, epidermal ingrowths projecting into the dermis were frequently seen at and after 2 months of UVB-irradiation. These findings indicate a high and rapid proliferation of keratinocytes.

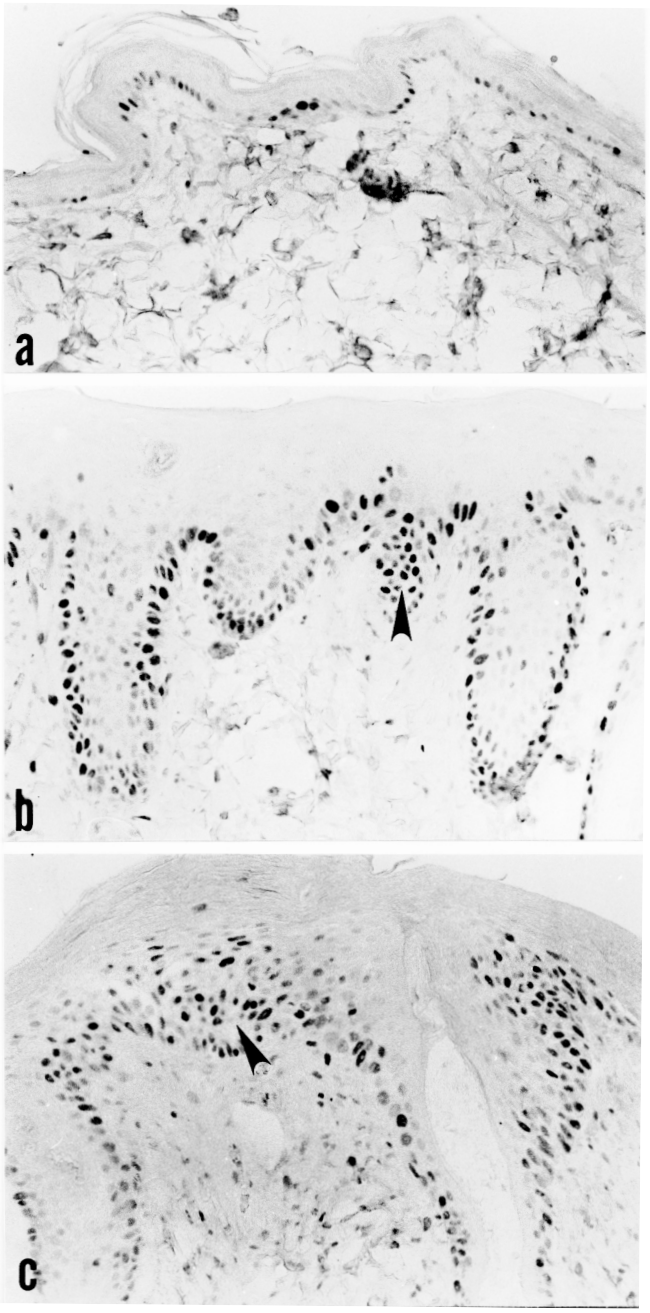

Fig. 8. Dorsal skin of a control rat **(a)**, and UVB-irradiated rats at 2 months **(b)** and 3 months **(c)**. As compared with a control skin **(a)**, many PCNA-positive cells are seen in the basal layer of epidermis including epidermal ingrowths (**b** and **c**). PCNA-positive cells are also seen within a small mass of keratinocytes forming small-sized epidermal ingrowths (arrowhead) **(b)** or showing a focal epidermal hyperplasia (arrowhead) **(c)**. Immunostaining for PCNA, x 175

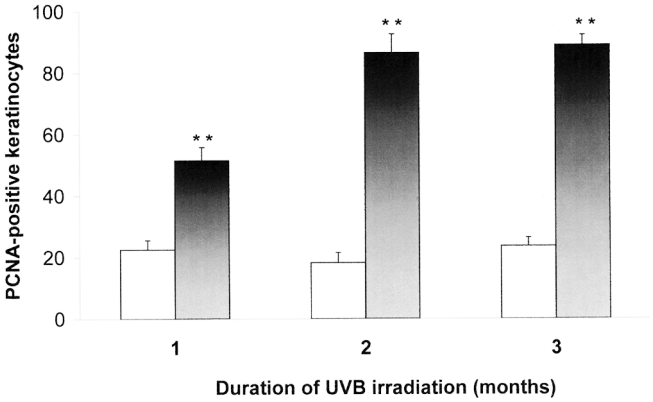

Fig. 9. Changes in the number of PCNA-positive cells in the dorsal skin epidermis of UVB-irradiated rats. Data are shown as mean ± SD of 3 (control group, black square) or 5 rats (UVB-irradiated group, white square). **: $p<0.01$, significantly different from control.

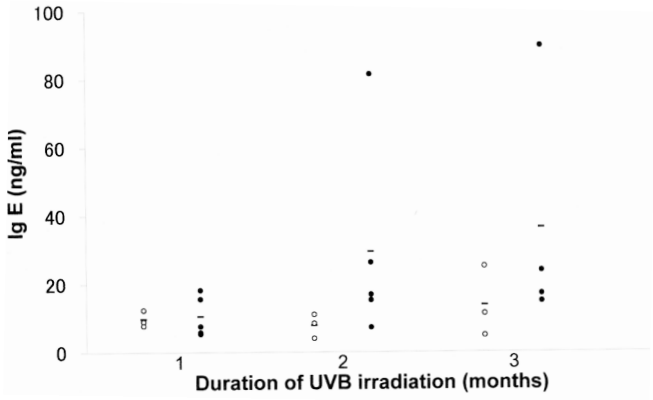

Fig. 10. Changes in the serum IgE concentrations in UVB-irradiated rats. Circles show individual values, and bars show mean of 3 (control group, white circle) or 4-5 rats (UVB-irradiated group, black circle).

In the control skin, PCNA-positive cells were found in the basal layer, and their number was about 20. In the case of UVB-irradiated skin, the number increased to around 90 PCNA-positive cells (at 3 months), and were also generally observed in the basal layer of the epidermis including the epidermal ingrowths and in the outer layer of the hair follicle epithelium. Moreover, immunoreactivity for PCNA was also found within a small mass of keratinocytes forming small-sized epidermal ingrowths or showing a focal epidermal thickening. In malignancies, the increase of PCNA is presumably associated with uncontrolled DNA synthesis and cell cycling, and therefore it is suggested that the percentage and distribution of PCNA-positive cells in the skin may be helpful in the early diagnosis of skin malignancies (Kawashira, 1999). Although it is obscure whether the present results of immunohistochemistry for PCNA suggest an early malignancy of keratinocytes or not, some interesting histological features were noted. Namely, keratinocytes, especially in the epidermal ingrowths, were somewhat pleomorphic. Moreover, in some portions, keratinocytes migrated into the dermis. These findings of keratinocytes may have a relation to a future development of skin cancer. In this connection, as compared with the control skin samples, the level of H-*ras* mRNA expression in the present UVB-irradiated skin samples measured by reverse the transcriptase-polymerase chain reaction (RT-PCR) method significantly increased with the day of UVB-irradiation, about which data will be published elsewhere. Increased expression and activation of H-*ras* was reported in relation with rat skin cancer induced by ionizing radiation (Maronpot, 1991). In addition, Mitchell et al. (1999) reported that DNA damage accumulated in mouse skin as a result of chronic irradiation and that this damage persisted in the epidermis and dermis for several weeks after the chronic treatment was terminated. They also assessed that skin cells exposed to UVB-irradiation over a long period of time may experience a DNA damage accumulation with mutagenic lesions that play an essential role in the onset of tumors (Mitchell et al., 2001). Parrish et al. (1981) also described that the effects of repeated subclinical but injurious exposures to UVB may constitute a significant fraction of cumulative or long-term actinic changes like wrinkling or carcinogenesis. Ultrastructural examinations of keratinocytes are now in progress to obtain more detailed morphological information.

In the present study, parakeratosis developed at 1 month of UVB-irradiation and progressed thereafter, and this may be related with a prominent and rapid proliferation of keratinocytes, as suggested by Benjamin et al. (1991).

Among the connective tissue components, collagen bundles in the upper dermis, especially beneath the epidermis, showed degeneration, and the thick bundles were dissociated into fine fibrils or broken down into small fragments. This may be related with intradermal edema and mechanical pressure due to epidermal thickening with prominent proliferation of keratinocytes.

Mast cells participate in a broader spectrum of biological processes including wound healing, angiogenesis, pathological fibrosis through activation and proliferation of fibroblasts, and host reaction to certain neoplasm (Hafez and Costlow, 1998; Meininger and Zetter, 1992; Claman, 1993). Mast cells are recognized as an important source of a variety of preformed and newly synthesized mediators such as histamin, heparin, proteases, and multifunctional cytokines (Galli et al., 1991; Schwartz, 1994). In the present study, the number of mast cells in the dermis prominently increased with the day of UVB-irradiation. At the same time, the concentration of serum IgE, although not significant, showed a tendency to increase at 2 and 3 months of UVB-irradiation. These findings may be responsible for the progression of intradermal edema and proliferation of fibroblasts.

In the present study, there were no changes in the serum IgG concentration or in the histology of the lymphoid tissues, although it is said that exposure of the skin to UV radiation results in immunosuppression (Nishimura et al., 1999). Further study on the effects of UVB-irradiation on T lymphocyte functions is now in progress.

In conclusion, subchronic UVB-irradiation induced a rapid and prominent keratinocyte hyperplasia, resulting in epidermal thickening and formation of epidermal ingrowths projecting into the dermis, and edema with capillary congestion, fibroblast proliferation, and increase in the number of mast cells in the dorsal skin of WBN/ILA-*Ht* rats. In addition, in some portions of the epidermis, especially in the epidermal ingrowths, keratinocytes were somewhat pleomorphic and migrated into the dermis.

References

Albarenque S.M., Shinozuka J., Iwamoto S., Nakayama H. and Doi K. (1999). T-2 toxin-induced acute skin lesions in Wistar-derived hypotrichotic WBN/ILA-Ht rats. Histol. Histopathol. 14, 337-342.

Ambach W. and Blumthaler M. (1993). Biological effectiveness of solar UV radiation in humans. Experientia 49, 747-753.

Anderson R.R. and Parrish J.A. (1981). The optics of human skin. J. Invest. Dermatol. 77, 13-19.

Benjamin S.A., Nikula K.J., Powers B.E. and Hahn F.F. (1991). Radiation and heat. In: Handbook of toxicologic pathology. Haschek W.M. and Rousseaux C.G. (eds). Academic Press Inc. San Diego. pp 983-1045.

Brash D.E., Rudolph J.A., Simon J.A., Lin A., McKenna G.J., Baden H.P., Halperin A.J. and Pontén J. (1991). A role for sunlight in skin cancer: UV-induced p53 mutations in squamous cell carcinoma. Proc. Natl. Acad. Sci. USA 88, 10124-10128.

Claman H.N. (1993). Mast cells and fibrosis: hints from graft-versus-host disease and scleroderma. In: The mast cell in health and disease. Kaliner M.A. and Metcalfe D.D. (eds). Dekker. New York. pp 653-667.

Devary Y., Rosette C., Didonato J.A. and Karin M. (1993). NF-κB

activation by ultraviolet light not dependent on a nuclear signal. Science 261, 1442-1445.

Galli S.J., Gordon J.R. and Wershil B.K. (1991). Cytokine production by mast cells and basophils. Curr. Opin. Immunol. 3, 865-872.

Haake A.R. and Polakowska R. (1993). Cell death by apoptosis in epidermal biology. J. Invest. Dermatol. 101, 107-112.

Hafez M.M. and Costlow M.E. (1988). Prolactin binding and localization in rat mammary tumor mast cells. Cancer Res. 48, 3765-3771.

Iwamoto S., Nakayama H., Yasoshima A. and Doi K. (1997). Hydrogen peroxide-induced dermatitis in WBN/ILA-Ht rats. Exp. Anim. 46, 147-152.

Jones T. (1997). The skin and its appendages. In: Veterinary pathology. 6th ed. Jones T.C., Hunt R.D. and King N.M. (eds). Williams & Wilkings. Baltimore. pp 817-871.

Kane K. and Maytin E. (1995). Ultraviolet B-induced apoptosis of keratinocytes in murine skin is reduced by mild local hyperthermia. J. Invest. Dermatol. 104, 62-67.

Kawashira K. (1999). Immunohistochemical staining of proliferating cell nuclear antigen (PCNA) in malignant and nonmalignant skin diseases. Arch. Dermatol. Res. 291, 413-418.

Kuroki K., Kimura T., Nakayama H. and Doi K. (1998). Manganese ethylene bis (Maneb)-induced degeneration of hair follicle epithelia in the dorsal skin of WBN/ILA-Ht rats. J. Toxicol. Pathol. 11, 205-207.

Kuroki K., Kimura T., Nakayama H. and Doi K. (2001). Acute dorsal skin responses to UVB-irradiation in Wistar-derived hypotrichotic WBN/ILA-Ht rats. Exp. Toxicol. Pathol. 53, 1-6.

Malcotti V., Yasoshima A., Uetsuka K., Nakayama H. and Doi K. (2001a). Early ultrastructural changes in the dorsal skin epidermis of Wistar-derived hypotrichotic WBN/ILA-Ht rats after UVB-irradiation. J. Toxicol. Pathol. 14, 173-177.

Malcotti V., Kuroki K., Nakayama H. and Doi K. (2001b). Effects of double UVB-irradiations with different intervals on the dorsal skin of Wistar-derived hypotrichotic WBN/ILA-Ht rats. Exp. Toxicol. Pathol. 53, 107-114.

Maronpot R.R. (1991). Chemical carcinogenesis. In: Handbook of toxicologic pathology. Hanschek W.M. and Rousseaux C.G. (eds). Academic Press Inc. San Diego. pp 91-129.

Meininger C.J. and Zetter B.R. (1992). Mast cells and angiogenesis. Semin. Cancer Biol. 3, 73-79.

Mitchell D.L., Greinert R., De Gruijl F., Guikers K., Breitbart E., Byrom M., Gallmeier M., Lowery M. and Volkmer B. (1999). Effects of chronic low dose ultraviolet B radiation on DNA damage and repair in mouse skin. Cancer Res. 59, 2875-2884.

Mitchell D.L., Byrom M., Chiarello S. and Lowery M.G. (2001). Effects of chronic exposure to ultraviolet B radiation on DNA repair in the dermis and epidermis of the hairless mouse. J. Invest. Dermatol. 116, 209-215.

Nishimura M. and Ishikawa S. (1987). Genetics of dominant hairless rats. Lab. Anim. 4, 13-14.

Nishimura N., Tohyama C., Satoh M., Nishimura H. and Reeve V.E. (1999). Defective immune response and severe skin damage following UVB irradiation in interleukin-6-deficient mice. Immunology 97, 77-83.

Ouhtit A., Konrad M.H., Davis DW., Ullrich S.E., McConkey D. and Anasthaswamy H.N. (2000). Temporal events in skin injury and the early adaptive responses in ultraviolet-irradiated mouse skin. Am. J. Pathol. 156, 201-207.

Parrish J.A., Zaynoun S. and Andreson R. (1981). Cumulative effects of repeated subthreshold doses of ultraviolet radiation. J. Invest. Dermatol. 76, 356-358.

Rice R.H. and Cohen D. (1996). Toxic responses of the skin. In: Casarett & Doull's toxicology. 5th ed. Klaassen C.D. (ed). McGraw-Hill. New York. pp 529-546.

Schwartz L.B. (1994). Tryptase: a mast cell serine protease. Methods Enzymol. 244, 88-100.

Young A. (1987). The sunburn cell. Photodermatology 4, 127-134

Zamansky G.B. and Chou I-N. (1987). Environmental wavelengths of ultraviolet light induce cytoskeletal damage. J. Invest. Dermatol. 89, 603-606.

Accepted January 9, 2002