UVB-INDUCED CHANGES OF BARRIER FUNCTION AND MORPHOLOGY OF THE HAIRLESS MOUSE SKIN

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자외선-B에의한 Hairless mouse의 보호기능과 표면구조의 변화 영향

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Abstract

Hairless mice (Skh:HR-1) exposed to single doses (0.5, 1.0 and 3.0MED) of UV-B radiation were displayed remarkable changes of barrier fuction and surface morphology. Trans- epidermal water loss (TEWL) as an index of barrier function was measured by evaporimeter, and wrinkle density (WD) as an index of morphological alteration was measured by image analyzer. Significant changes of TEWL were not observed in the control and 0.5MED group, but 1.0MED and 3.0MED groups noted significant difference. TEWL of 3.0MED group was rapidly increased to the 3rd day and decreased until the 14th day when it reached nearly to normal level. Time-courses of TEWL for 1.0MED and 3.0MED groups displayed similar pattern, but different only in the magnitude. WD were significantly decreased during the 3rd-5th day in all of the irradiated groups and then increased during the last period to the 14th day, but did not recover the normal level at the 14th day. Time-courses of WD

for all groups exhibited similarity, and were entirely dependent on the exposed doses. We also observed histological changes which included hyperplasia, sunburn cell (SBC) formation, accumulation of polymorphonuclear leukocyte (PMNs), and loss of collagen of UVB-exposed hairless mouse skin. Changes of TEWL and WD are helpful in understanding of epidermal and dermal damages by single exposure of UVB.

INTRODUCTION

UltravioletB(UVB) radiation is responsible for many of acute and chronic effects of solar exposure, such as histological, physical and visible changes in relation to the photoaging and photodamages (1,2,3,4,5). Many groups reported these changes which were induced by epidermal and dermal damages. In a recent study, the acute effects of UVB were mainly concerned with the changes in the epidermal lipid barrier and the surface morphology of UVB irradiated hairless mouse skin (6).

Lipid barrier in Stratum corneum was consisted of ceramides (45.8%), free fatty acids (13.1%), cholesterol (26%), cholesteryl sulfate (3.9%), unidentified components (5.7%), and so on (7), and it served as a bound-water modulator (8,9), and a regulator of permeability (9,10). Formation of sunburn cell(SBC) (5,11,12,13) and hyperkeratinization (14,15) in UVB-induced human, mouse and rat skin produced the early desquamation (14), defect of corneocyte(16) and decomposition of lipids (17,18). TEWL is increased as a result of responses in epidermis.

The vasodilatory effect of UVB on the skin vasculature resulted in increased blood flow (13,19,20,21), and PMNs were accumulated in the lesion (22,23).

Many proteolytic and hydrolytic enzymes, such as collagenase, elastase and cathepsin are excreted from accumulated PMNs after UVB exposure (22,23). The degradation of the collagenous component of the extracellular matrix was caused by the specific cleavage of type III collagen by purified PMNs elastase (33). Morphological changes of skin surface was induced by such dermal degradation(12,21). Many groups reported on the chronic effect of UVB exposed hairless mouse (1,2,3,24, 25,26). Until recently, acute effect of UVB single exposure has been afforded less attention. The purpose of this paper is to monitor, for 2 weeks, the acute effects of a single exposure to UVB non-invasively by comparing clinical observations with evapometry and image analysis.

Additionally, we observed the histological changes of epidermis and dermis in the UVB irradiated hairless mouse skin.

MATERIALS AND METHODS

Animals Ninty-six female hairless mice (Skh:HR-1), aged 12-15 weeks were obtained from Charles River Laboratories (Willington, MA). Animals were acclimated (Temp.:24 ± 1℃, Humid.:50 ± 3%RH) for 1 week prior to experiment.

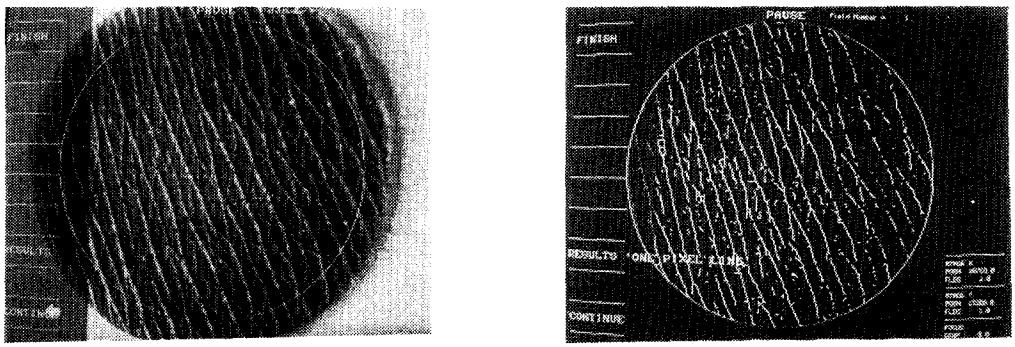
UVB Radiation Source and Schedules Test animals were divided into four groups (Non-irradiated, 0.5MED, 1.0MED and 3.0MED ; six

animals per group) and were exposed to UVB radiation (290-320nm), from a bank of unfiltered UV-B313 lamps (40W, Q-Panel co.,USA) placed 28cm above the backs (2.5×3.5cm) of the mice. Irradiance was measured with an IL1700 radiometer (International Light) which exhibits peak sensitivity at 296nm (Detector:SED 240). Before experiments, MED(1MED = 28 ± 3mJ/cm^2, n=6) was determined 24h after a single UVB exposure. The mesurements of TEWL and WD were done at the 0, 1st, 3rd, 5th, 7th and 14th day after UVB exposure, and performed under controlled conditions (Temp.:24 ±1℃, Humid.:50 ± 3%RH).

TEWL Measurement Twenty-four animals were tested. Transepidermal water loss (TEWL) was measured with an Evaporimeter EP1 (Servo Med,Sweden) (27), and taken mid-way between the shoulders and hips. The TEWL value, expressed in g/m^2/h, was registered on a pen recorder until a stable level was reached, usually within 1min.

Wrinkle Density Measurement Forty-eight animals were tested. At each time point, Mouse skin impressions were made using a Xantopren^R brand silicone elastic impression material (Bayer, Germany) to the excied backs of mice. We obtained negative Xantopren^R replicas on a circular patch of 12mm internal diameter. A small tongue was used as a mark for alignment with respect to the body axis. Each sample (replica plus adhesive) was glued to a black metallic holder which ensured that the field to be analysed was plane. The holder was then inserted in a rotary system and centered under the chalnicon head of a Quantimet 970 (Cambridge Instru., UK). When the

skin replica was irradiated by the 26° incident light, the shadows were acquired by the wrinkles of replica surface, and trasformed into the "one pixel line" by the "skeleton mode(cycle = 30)" of image analysis software (Fig. 1). The sum of "one pixel line" in a fixed area represented wrinkle density (WD) which was described by Corcuff *et al.* (28) as the "line density", and it meant the degree of skin wrinkles. Primary and secondary WD were measured to parallel and perpendicular body axis.



a b

Fig. 1 a:Photograph of the replica (20x)
b:Photograph of the transformed "one pixel line"

Histology Eighteen animals were tested. With UVB-irradiated and non-irradiated mice, histological observations were done at the 0, 3rd and 14th day. Three mice from each group were sacrified at each time point for histology. Strips of dosal skin were fixed in 10% buffered formalin, embedded in paraffin, and sectioned at 4μm. Sections were stained with hematoxylin and eosin (H&E) for SBC, Masson's Trichrome for collagen.

Statistical methods Student's t-test was employed to evaluate the result.

RESULTS

TEWL Fig. 2 shows the time courses of TEWL. Significant changes of TEWL were not observed in the control and 0.5MED groups, but 1.0MED and 3.0MED groups noted significant differences (1.0MED: $p<0.05$ and 3.0MED: $p<0.001$). TEWL of 3.0MED group was rapidly increased to the 3rd day and decreased during the last periods until the 14th day which reached nearly to normal level. Time-courses of TEWL for 1.0MED and 3.0MED groups displayed similar pattern, but defferent only in the magnitude.

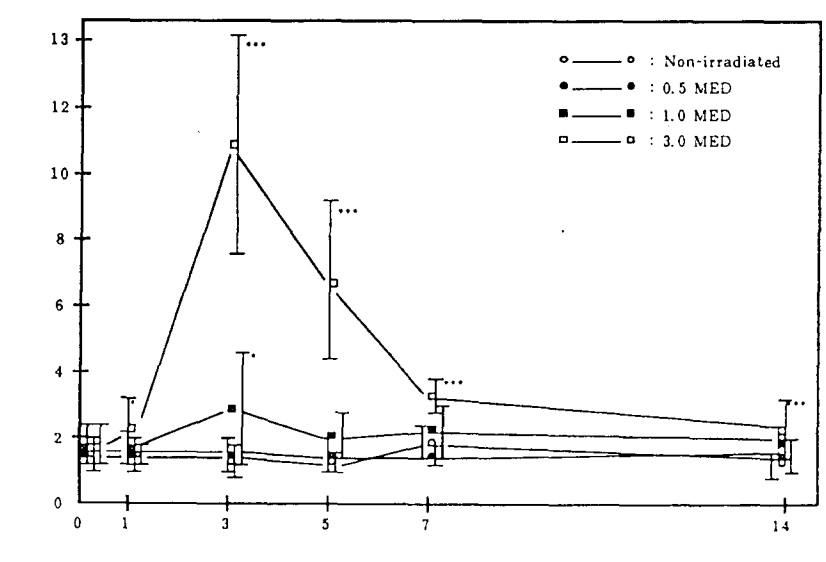


Fig. 2 Time courses of TEWL, Mean±SD(*: $p<0.05$, ***: $p<0.001$, compared with control, n=6)

Wrinkle Density Fig. 3, Fig. 4 and Fig. 5 show the time courses of primary, secondary, and primary plus secondary WD. WD were significantly decreased during the 3rd-5th day in all of the irradiated groups and then increased during the last period to the 14th day, but did not recover the normal levels at the 14th day. Time-courses of WD for all groups exhibited very similar patterns, and they were entirely dependent on the exposed doses. Fig. 3 shows the time course of primary WD. WD of 0.5MED group decreased to the 3rd day and then slowly increased during the last period until the 14th day. It was a typical curve for all of time courses including 1.0MED and 3.0MED groups. Fig. 4 and Fig. 5 show the time courses of secondary WD and primary plus secondary WD respectively. The curves, time courses of WD, showed very similar features. WD values of all of the groups were decreased significantly from the date of UVB irradiation to the 3rd-5th day, increased toward the normal level (Fig. 3, 4, 5).

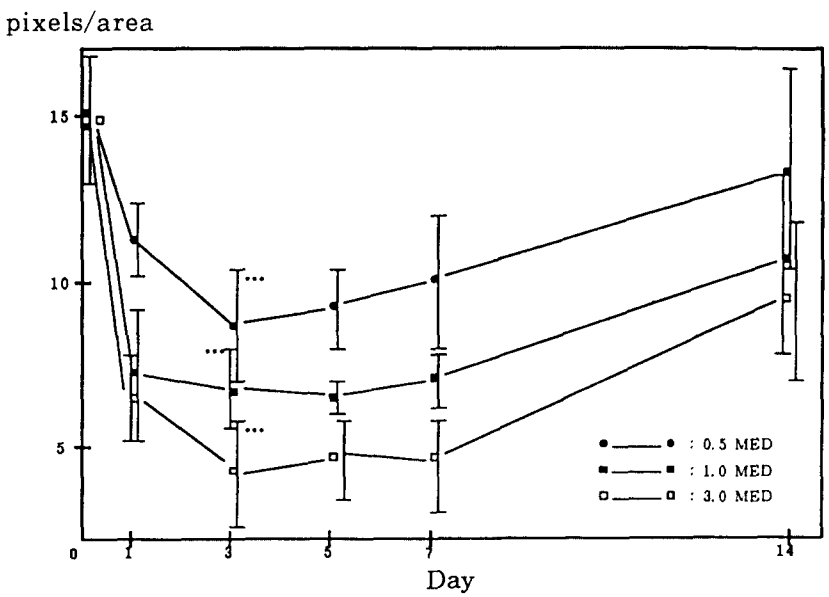


Fig. 3 Time courses of Primary WD, Mean±SD(*:p<0.05, ***:p<0.001, compared with non-irradiated)

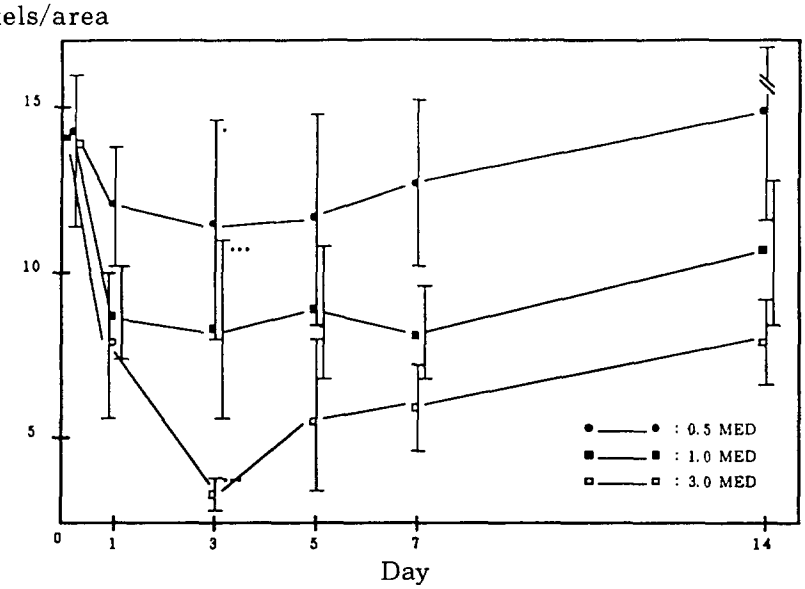


Fig. 4 Time courses of Secondary WD, Mean±SD(*:p<0.05, ***:p<0.001, compared with non-irradiated)

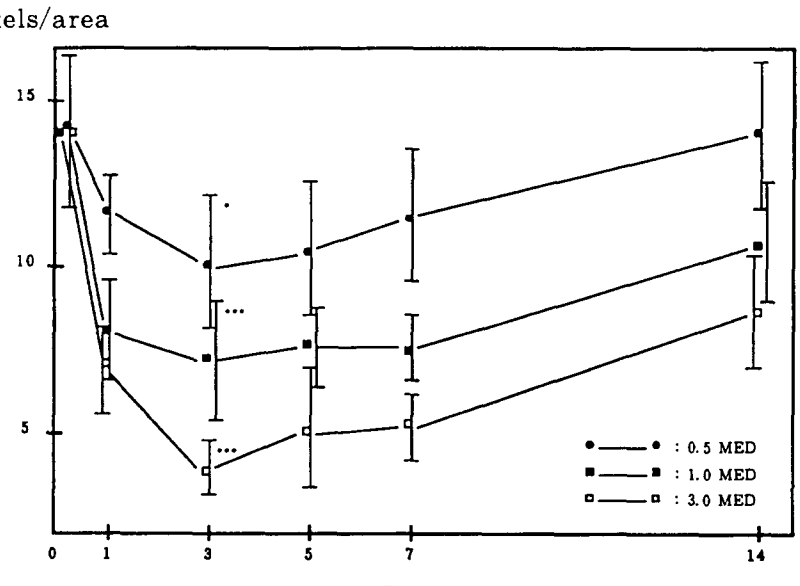
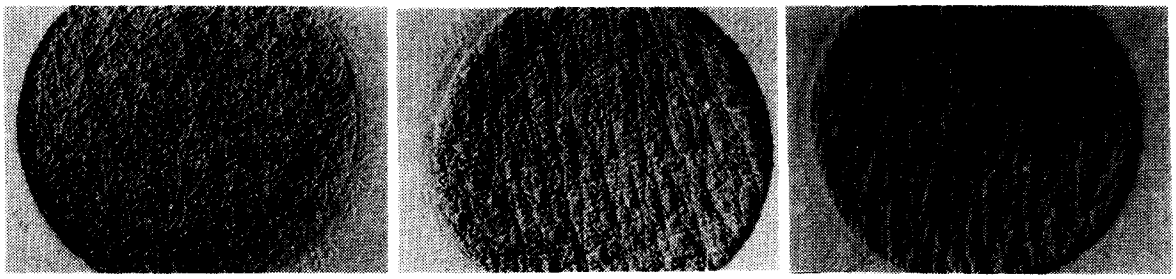
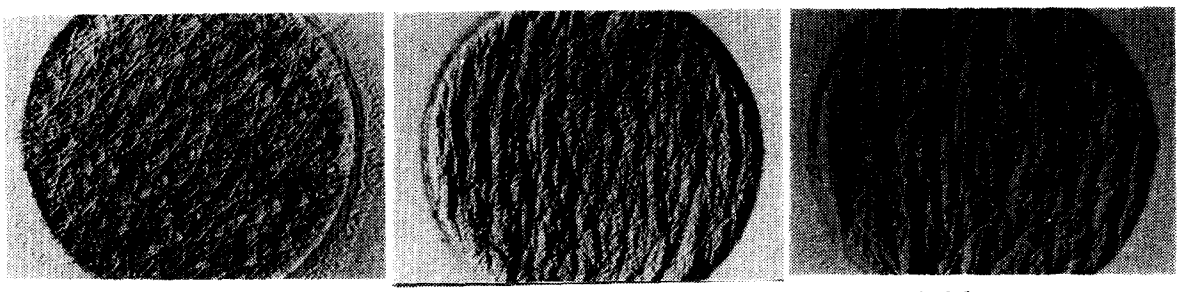


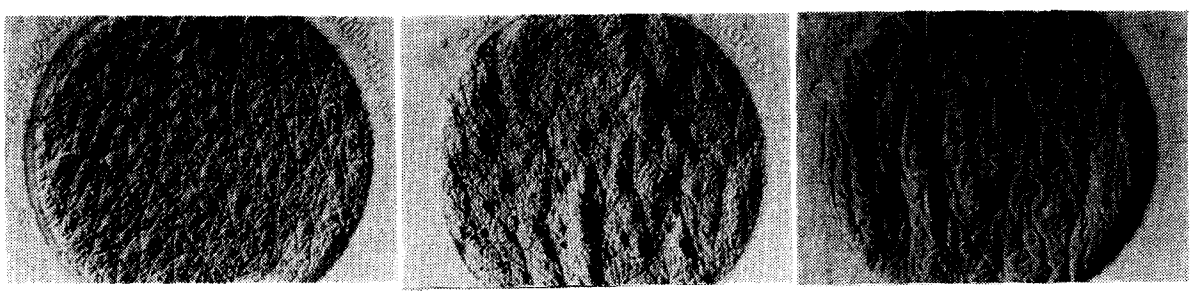
Fig. 5 Time courses of Primary plus Secondary WD, Mean±SD (*:p<0.05, ***:p<0.001, compared with non-irradiated)



a:before irradiation b:1day c:3day



g:before irradiation h:1day i:3day



m:before irradiation n:1day o:3day

Fig. 6 Photographs of skin replicas ; a-f:0.5MED, g-l:1.0MED and m-r:3.0MED.

Fig. 6 shows the changes of wrinkling according to experimental period and doses of UVB. The primary wrinkles become more obvious, deeper and wider until the 3rd-5th day. Like WD, wrinkles on the replicas exhibited the similar trends that showed severe wrinkles during the 3rd-5th day and recovering process till the 14th day, visibly.

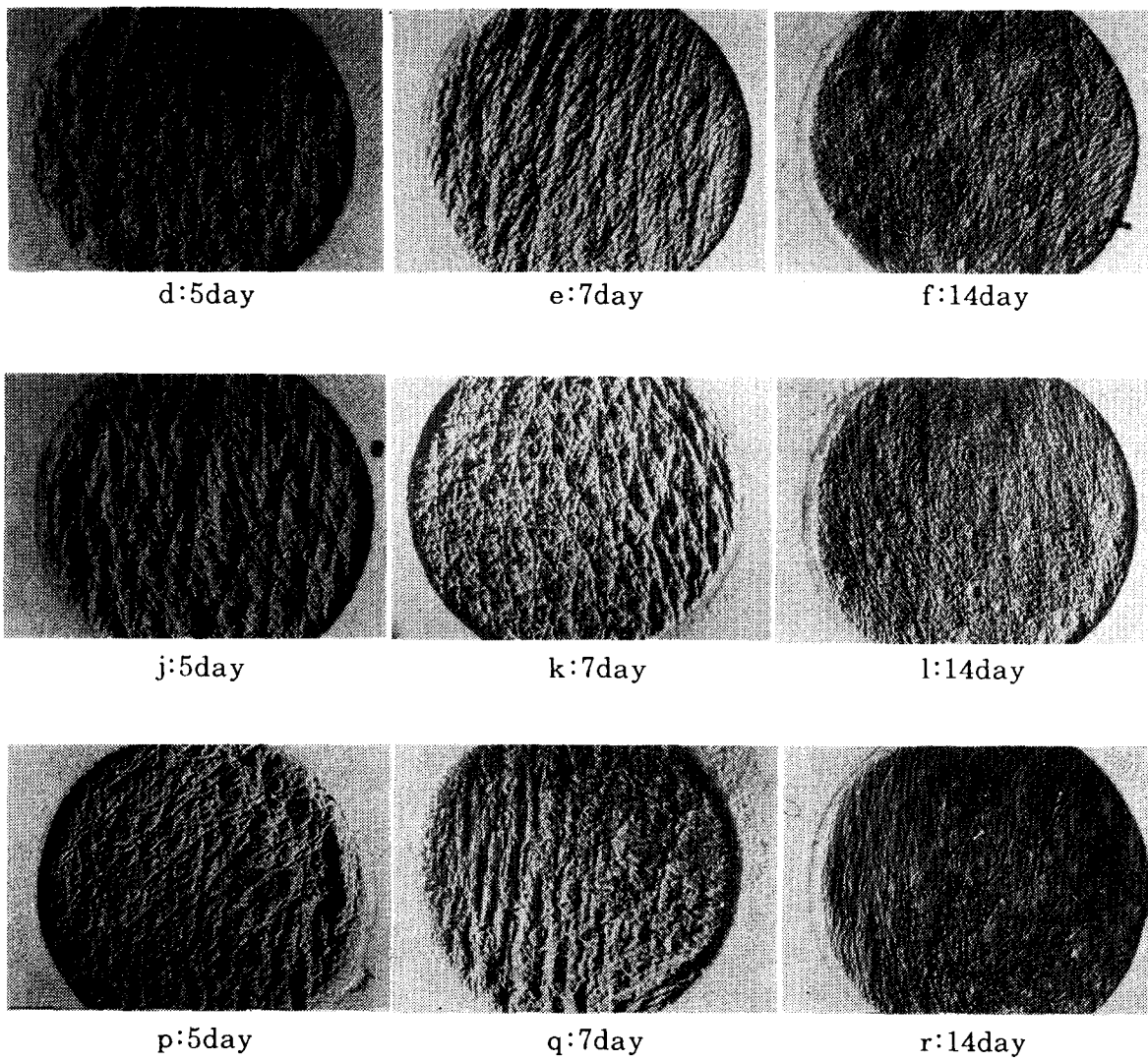


Fig. 6 (continue)

In the secondary wrinkles, they were gradually shallowed and disappeared until the 3rd-5th day. After the 3rd-5th day, they were recovering toward the normal features.

Histology Fig. 7(a, b, c) showed the changes of irradiated epidermis during the experimental period. Hyperplasia and SBC formation of the epidermis reached to the maximum peak at the 3rd day. The greatest infiltrate of PMNs and loss of collagen were also observed in the dermis of biopsies from 3.0MED exposed mice at the 1st (Fig. not presented) and 3rd day, respectively (Fig. 7 d, e, f).

a:before irradiation b:3day c:14day

d:before irradiation e:3day f:14day

Fig. 7 Histological changes of 3.0MED exposed hairless mice ;
a-c : for hyperplasia and SBC (H&E, x64, Leitz Metallux 3),
d-f : for collagen and PMNs (Masson's Trichrome, x64)

DISCUSSION

The short-term effects of single exposure to hairless mouse have been afford less attention.

Bisset et al (2) have shown that chronic exposure of hairless mice to UVB radiation alone or a combination of UVA and UVB radiation permanently alters TEWL. Elevated TEWL values indicate damage to the barrier such as many occur after tape stripping (29), in irritant dermatitis (30), and in dermatoses such as psoriasis (31). In one study, increasing UV dose led to a concomitant increase in transepidermal water loss of hairless rat skin. Using UVB irradiation in hairless rats as a model, Lamaud (14) observed a peak of the TEWL at 72h after UV irradiation when increased desquamation. We also observed very similar results from hairless mice skin (Fig. 2). But in a previous work for human skin (6), no increase in TEWL could be recorded by evaporimetry except on the area irradiated with a single dose of 3.0MED UVB, where 4 persons among 6 persons showed a moderate increase after 2 weeks. According to our observation, the thickness of epidermis of hairless mouse (15-25μ m, Fig.7) was thinner than that of human (40-50μ m (32)). Thinner epidermis may induce the immediate response for TEWL in hairless mouse skin, because it is more susceptible for UVB radiation.

Bissett et al (2,3) reported the surface wrinkling by the chronic exposure, but we had many difficulties to find the reports for the acute exposure. Fig.3 clearly demonstrates the great similarity in morphological response to UVB, all of which produced maximal changes

after the 3-5 days. WD were significantly decreased during the 3rd-5th day in all of the irradiated groups and then increased during the last period to the 14th day, but did not recover the normal level at the 14th day. Visibly, Fig.6 shows the entire trends, and we can find the correlation between UVB doses and the degree of wrinkling. More importantly, the recovering rate was independent of the exposed doses, and exhibited the constant slope for WD vs. day (Fig.3,4,5). Understanding of dermal changes needs more studies on the recovering rate which relate to the histological changes.

Of the time interval used here, maximum histological changes in epidermis and dermis were present at the 1st and 3rd day. Rosario et al. (15) followed the histological changes induced by 3-6MED by human biopsy 1, 2, 3, and 7 days after a single exposure, and found hyperkeratosis at the 3rd day on the areas exposed to UVB and UVC but not on those treated with UVA. Our observations for hyperkeratosis and hyperplasia in 3.0MED irradiated hairless mouse are similar to their results in human biopsy (Fig.7 a,b,c). The appea-rance and the number of SBC were clearly dependent on the dose of UVB radiation and the time interval after exposure (data not presented). Additionally, the accumulation of PMNs and the loss of collagen were observed at the 1st and 3rd day on the areas exposed to UVB, respectively (Fig.7 d,e,f).

The rate of TEWL reflects the function of the inter-cellular lipid-barrier in stratum corneum, and evaporimetry is a sensitive indicator of this function (29). In case of morphological changes, wrinkles of skin surface reflect the dermal damage, and measurement of WD, our method, is thought to be more sensitive indicator of this damage.

Especially, WD measurement might be more preciser method to study the effects of suberythemal exposure than any other methods.

All of the responses for TEWL, WD and histological changes except for the accumulation of PMNs exhibited a peak for the maximum, it suggests that the changes of the 3rd day is very important for understanding of UVB-induced epidermal and dermal changes.

요 약

Hairless mouse에 자외선-B를 일회조사한후 시간에따른 피부보호기능과 표면구조변화의 정도를 TEWL과 주름살밀도로 측정하였다. 자외선-B의 조사 량은 0.5, 1 과 3 MED 였으며, 측정 및 관찰은 조사전과 조사후 1, 3, 5, 7 및 14일에 수행하였다. TEWL은 Evaporimeter로, 주름살밀도는 Silicone replica와 Image-analyzer를 이용하여 측정하였다. 조사전과 0.5MED 그룹들의 TEWL 값은 변화가 없었으나, 1 과 3MED그룹들의 TEWL값은 현저 한 변화를 보여 3일째에 극대값을 보였고, 이후 감소하기 시작하여 14일째에는 거의 정상값에 도달하였다. 반면 주름살밀도는 자외선-B의 조사량에 비례하는 경향을 보여 모든 조사그룹에서 3-5일째까지 감소한후 서서히 증가하였다. 결 과로부터, 1) 자외선조사후 시간에따른 TEWL과 주름살밀도의 최대·소값은 3일째 나타났으며, 2) 주름살밀도의 회복정도가 조사량에 상관없이 시간에따라 일정한 기울기를 보였으며, 3) 주름살밀도측정만이 Sub-erythemal 손상의 정도차를 유의하게 보여주었다

REFERENCES

1. L.H. Kligman, F.J. Akin and A.M. Kligman, Prevention of Ultraviolet Damage to the Dermis of Hairless Mice by sunscreen. J

Invest Dermatol 78:181-189, 1982

2. D.L. Bissett, D.P. Hannon and T.V. Orr, An animal model of solar-aged skin: Histological, Physical, and Visible changes in UV-irradiated Hairless mouse skin. Photochem Photobiol Vol 46, No 3: 367-378, 1987
3. I. Kiss, S. Chen and K.M. Tramposch, The effect of high and low Ultraviolet-B dose exposure on the degree of Hairless mouse skin wrinkling. Photochem Photobiol Vol 53, No 1:109-112, 1991
4. E. Schwartz, Connective tissue alterations in the skin of ultraviolet irradiated hairless mice. J. Invest. Dermatol. 91:158-161 1988
5. K.H. Kaidbey, G.L. Grove, and A.M. Kligman, The influence of longwave ultraviolet radiation on sunburn cell production by UVA. J. Invest. Dermatol. 73:243-245 1979
6. T. Frodin, L. Molin and M. Skogh, Effect of single dose of UVA, UVB, and UVC on skin blood flow, water content, and barrier function measured by laser-Doppler flowmetry, optothermal infrared spectrometry, and evaporimetry. Photodermatology 5:187-195, 1988
7. D.C. Swartzendruber, P.W. Wertz, K.C. Madison and D.T. Downing, Evidence That the Corneocyte has a Chemically Bound Lipid Envelope. J Invest Dermatol 88:709-713, 1987
8. G. Imokawa, H. Kuno and M. Kawai, Stratum Corneum Lipids Serve as a Bound-Water Modulator. J Invest Dermatol 96:845-851, 1991
9. D.T. Downing, Lipids:Their role in epidermial structure and function. Cosmetics & Toiletries 106:63-69, Dec. 1991
10. P.M. Elias, Structure and Function of the Stratum Corneum Permiability Barrier. Drug Development Reasearch 13:97-105, 1988

11. A. Woodcock and I.A. Magnus, The sunburn cell in mouse skin: preliminary quantitative studies on its production. Br J Dermatol 95: 459-468, 1976

12. P.G. Agache, E. Quencez, The mechanism of solar erythema. J. Appl. Cosmetol. 6:69-78 1988

13. T.B. Fitzpatrick, M.A. Pathak, L.C. Harber, M. Seiji and A. Kukita, Sunlight and Man, p143-146, 1977, University of Tokyo Press

14. E. Lamaud and W. Schalla, Influence of UV irradiation on penetration of hydrocortisone. In vivo study in hairless rat skin. Br J Dermatol Vol 111, supplement 27:152-157, 1984

15. R. Rosario, G.J. Mark, J.A. Parrish and M.C. Mihm, Histological changes produced in skin by equally erythemogenic dose of UV-A, UV-B, UV-C and UV-A with psoralens, Br J Dermatol 101:299-308, 1979

16. N. Kashibuchi, Improved Exfoliative Cytology for Morphological Evaluation of Skin. 15th IFSCC International Congress paper No.13: 177-196, 1988

17. H. Wefers, B.C. Melnik, M. Flur, C. Bluhm, P. Lehmann and G. Plewig, Influence of UV Irradiation on the Composition of Human Stratum Corneum Lipids. J Invest Dermatol 95:959-962, 1991

18. F. Chatenay, P. Corcuff, D. Saint - Leger and J.L. Leveque, Alterations in the composition of human stratum corneum lipids induced by inflammation. Photodermatol Photoimmunol Photomed 7: 119-122, 1990

19. A.R. Young, R.H. Guy and H.I. Maibach, Laser Doppler Velocimetry to Quantify UV-B Induced Increase in Human Skin Blood Flow.

Photochem Photobiol Vol 42, No 4:385-390, 1985

20. P.M. Farr and B.L. Diffey, The vascukar response of human skin to ultraviolet radiation. Photochem. Photobiol. 44:501-507, 1986

21. B.A. Gilchrest, N.A. Soter, J.S. Stoff and M.C. Mihm, J Am Acad Dermatol 5:411-422, 1981

22. G.B. Ryan and G. Majno, Acute inflammation: A review. Am J Pathol Vol 86, No 1:184-276

23. A.K. Balin and A.M. Kligman, Aging and the Skin, p331-346, 1989, Raven Press, 1185 Avenue of the Americas, New York, New York 10036

24. S.R. Plastow, J.A. Harison, and A.R. Young, Early changes in dermal collagen of mice exposed to chronic UVB irradiation and the effects of a UVB sunscreen. J. Invest. Dermatol. 91:590-592 1988

25. S.R. Plastow, C.R. Lovell, and A.R. Young, UVB-induced collagen changes in the skin of the hairless albino mouse. J. Invest. Dermatol. 88:145-148 1987

26. R. Hirose, and L.H. Kligman, An ultrastructural study of ultraviolet-induced elastic fiber damage in hairless mouse skin. J. Invest. Dermatol. 90:697-702 1988

27. J. Pinnagoda, R.A. Tupker and J. Serup, Guidelines for transepidermal water loss (TEWL) measurement. Contac Dermatitis 22:164-178, 1990

28. P, Corcuff, J. de Rigal and J.L. Leveque, Skin relief and aging. J Soc Cosmet Chem 34, 177-190, 1983

29. T. Frodin and M. Skogh, Measurement of Transepidermal Water Loss Using an Evaporimeter to follow the Restitution of the Barrier Layer of Human Epidermis after Stripping the Stratum corneum.

Acta Derm Venerol (Stockh) 64:537-540, 1984

30. T. Frodin and C. Andersson, Multiple parameter asse-ssement of skin irritancy. Contact Dermatitis 17:92-99, 1987

31. T. Frodin, M. Skogh and L. Molin, The importance of the coal tar bath in the Ingram treatment of psoriasis. Evaluation by evaporimetry and laser Doppler flowmetry. Br J Dermatol 118:429-434, 1988

32. M.W. Greaves and S. Shuster ed, Handbook of Experimental Pharmacology Vol87/I:p3-30, 1989,

33. C.L. Malnardi, D.L. Hasty, J.M. Seyer and A.H. Kang, Specific cleavage of Human Type III Collagen by Human Polynuclear Leukocyte Elastase. J Biol Chem Vol 255, No 24:12006-12010, 1980