Effect of Protein Malnutrition on the Skin Epidermis of Hairless Mice

Akihiko SUGIYAMA1),*, Yuka FUJITA1), Toshihiro KOBAYASHI2), Mizuyuki RYU2), Yasushi SUZUKI2), Aino MASUDA3), Tairin OCHI3) and Takashi TAKEUCHI1)

1) Course of Veterinary Laboratory Medicine, School of Veterinary Medicine, Faculty of Agriculture, Tottori University, 4–101 Koyama-Minami, Tottori, Tottori 680–8553, 2) Biochemical Laboratory, Saraya Co., Ltd., 24–12 Tamate-cho, Kashihara, Osaka 582–0028 and 3) Japan Institute for the Control of Aging (JalCA), Nikken Seil Co., Ltd., 710–1 Haruoka, Fukuroi, Shizuoka 437–0122, Japan

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ABSTRACT. The purpose of this study was to evaluate the effect of protein malnutrition on the skin epidermis of mice. A low protein diet induced thinning of the skin epidermis, a decrease of cell proliferative activity in epidermal cells and a decrease of stratum corneum hydration. Dityrosine was immunostained in the cytoplasm of epidermal cells in the low protein diet group. Plasma advanced oxidation protein product (AOPP) levels were significantly more increased in the low protein diet group than in the control diet group. These results suggest that protein malnutrition adversely affects the structure and water barrier and reservoir functions of the skin epidermis, and these pathological changes are associated with the expressions of protein oxidation markers, dityrosine and AOPP.

KEY WORDS: advanced oxidation protein product, dityrosine, protein malnutrition, skin epidermis.

NOTE Laboratory Animal Science

Protein malnutrition (PM) is a common problem worldwide, occurring in both developing and industrialized countries. It is especially prevalent in children in developing countries. In industrialized countries, PM is often secondary to chronic diseases such as AIDS and cancer and mostly affects the hospitalized and the elderly [15]. PM affects the metabolism of several body proteins, resulting in the malfunction in a number of body parts, including the skin. Inappropriate protein nutrition can thus cause, for example, retardation of wound healing and deterioration of bedsores [23, 24]. Deterioration of the skin such as that occurring with epilation and achromia is one of the serious symptoms of kwashiorkor [29, 33]. PM affects the status of dermal collagen in humans and experimental animals. Oishi et al. [25] reported that the synthesis and degradation of types I and III collagen were affected by protein deficiency. However, to our knowledge, there have been no reports about the effect of PM on the skin epidermis.

Experimental and clinical data suggest that PM is associated with free radical damage [14, 28]. The erythrocyte glutathione concentrations were drastically reduced in kwashiorkor patients and were clearly related to the clinical outcome [10]. Children with kwashiorkor experienced a significant increase in urinary protein oxidation markers such as dityrosine and ortho-tyrosine [22]. In protein malnourished rats, the activities of antioxidant enzymes such as superoxide dismutase, glutathione peroxidase and catalase were decreased with a concomitant increase of lipid peroxidation in the liver, intestine and brain [5, 9, 11, 28]. However, it remains unclear whether protein oxidation markers such as dityrosine and advanced oxidation protein products (AOPPs) are associated with the pathological changes of the skin epidermis with PM.

Dityrosine has recently been developed as a biomarker of protein oxidation [2, 12, 20]. The oxidation of tyrosyl radical, and dityrosine is then formed by the reaction of two tyrosyl radicals [2, 12, 20]. Dityrosine is formed by reactive oxygen species (ROS), enzymatic reactions, ultraviolet irradiation and lipid peroxidation [16, 17, 19, 21]. AOPPs are defined as dityrosine-containing cross-linked protein products and are considered to be reliable markers for estimating the degree of protein oxidation [3, 8, 35].

The purpose of this study was to evaluate the effect of PM on the skin epidermis of hairless mice and the possible association between the pathological changes of the skin epidermis and the expression of protein oxidation markers, dityrosine and AOPPs.

Murine diets for use in this study were prepared in our laboratories. While both the low protein and control diets contained fiber, mineral mixtures (Oriental Yeast Co., Ltd., Tokyo, Japan) and balanced vitamin mixtures (Oriental Yeast Co., Ltd.) in the same quantity, the protein quantities in the two differed: the control purified diet contained 200 g/kg protein, and the hypoproteinemic purified diet contained only 20 g/kg [6]. Except for the protein content, the two diets were identical and isocaloric (Table 1).

All experiments were performed on 8-week-old, male HR-1 hairless mice (Hoshino Laboratory Animals, Corp., Ibaraki, Japan). The animals were maintained at a controlled temperature of 22 ± 2°C with a 12:12-hr light/dark cycle (light cycle, 07:00–19:00). The use of these animals and the procedures performed on them were approved by the Animal Research Committee at Tottori University.

A total of 12 animals were divided into two different groups as follows: (1) a control diet-feeding group (n=6) and (2) a low protein diet feeding group (n=6). All animals...
were fed each diet for 4 weeks. Blood and dorsal skin samples were collected under pentobarbital anesthesia (100 mg/kg, intraperitoneal injection). Plasma albumin levels were determined by means of a biochemical autoanalyzer (Dri-Chem 3000; Fujifilm Corporation, Tokyo, Japan). Plasma AOPP concentration was assayed by a colorimetric method with a commercial kit (Cell Biolabs, Inc. San Diego, CA, U.S.A.). Stratum corneum hydration was measured using Corneometer CM825 (Courage and Khazaka Electronics, Cologne, Germany).

Skin tissues were fixed in 10% buffered formaldehyde, processed for histological examination by the conventional methods and stained with hematoxylin and eosin (HE). Those conducting the histopathological examinations were blinded to the study treatments. Epidermal thickness was measured with histometric analysis software (Olympus Corporation, Tokyo, Japan).

For immunohistochemistry, the following primary antibodies were used: anti-dityrosine mouse monoclonal antibody (Nikken Seil Co., Ltd., Shizuoka, Japan) and Ki-67 antigen mouse monoclonal antibody (Dako, Tokyo, Japan). All sections were dewaxed, rehydrated, rinsed with 0.05 M tris-buffered saline (TBS; pH 7.6), treated with 3% hydrogen peroxide and then rinsed again with TBS. Tissue sections for detection of Ki-67 antigen were immersed in 0.01% hydrogen peroxide to facilitate a peroxidase color reaction. After a further wash with TBS, the slides were counterstained with Mayer’s hematoxylin. The cell proliferation index of the epidermis was determined as a percentage of epidermal cells with Ki-67 antigen [13].

All data are expressed as means ± SE of all mice in both groups. The results in both groups were compared by Student’s t-test. *P<0.05 was considered to be statistically significant.

As a result of low protein diet feeding for 4 weeks, body weight loss and decrease of body length and the plasma albumin concentration were observed (Table 2), and this was similar to the results of previous studies [1, 27]. The plasma BUN and creatinine concentrations in the low protein diet group were as low as those in the control diet group, and there were no statistical differences between the two groups (control diet group, BUN 28.1 ± 1.13 mg/dl, creatinine 0.13 ± 0.02 mg/dl; low protein diet group, BUN 23.0 ± 0.79 mg/dl, creatinine 0.15 ± 0.02 mg/dl). The stratum corneum hydration in the low protein diet group was significantly lower than that in the control diet group (Table 2).

Histopathological examination revealed a decrease in the thickness of the epidermis in the low protein diet group (Fig. 1). Indeed, atrophy of stratum granulosum was clearly observed in the epidermis (Fig. 1). These histopathological changes were observed in all mice of the low protein diet group. The cell proliferation index of the low protein diet group was decreased significantly in comparison with that of the control diet group (Fig. 1). There were no significant histopathological changes in both kidneys in the low protein diet group.

Epidermal cells in the control diet group were devoid of dityrosine. In all mice of the low protein diet group, however, dityrosine immunostained the cytoplasm of epidermal cells (Fig. 1). In addition, the low protein diet group showed a significant increase in plasma AOPP levels compared with the control diet group (Fig. 2).

These results revealed that the low protein diet induced the thinning of the epidermis, a decrease of proliferative activity in epidermal cells and a decrease of stratum corneum hydration. This study thus made it clear that protein malnutrition had a negative impact on the structure and water barrier and reservoir functions of the skin epidermis. It is likely that the decrease of stratum corneum hydration resulted from thinning of the epidermis.

PM reportedly impairs epithelial proliferation in the crypt of the small intestine, resulting in delayed cellular migration.

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### Table 1. Composition of the experimental diets*

<table>
<thead>
<tr>
<th></th>
<th>Control diet</th>
<th>Low protein diet</th>
</tr>
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<tbody>
<tr>
<td>Casein (&gt;85% protein)</td>
<td>200</td>
<td>20</td>
</tr>
<tr>
<td>Sucrose</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Fiber</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Corn oil</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>Mineral mixture†</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Vitamin mixture†</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>L-Methionine</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>556.5</td>
<td>736.5</td>
</tr>
</tbody>
</table>

* Isocaloric diets providing 1716.3 kJ/100 g (410.6 kcal/100 g).
† Mineral and vitamin mixtures were prepared according to the 1993 recommendations of the American Institute of Nutrition for adult mice [30].

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### Table 2. Effect of protein malnutrition on body weight, body length, plasma albumin and stratum corneum hydration

<table>
<thead>
<tr>
<th></th>
<th>Body weight (g)</th>
<th>Body length (cm)</th>
<th>Plasma albumin (g/dL)</th>
<th>Stratum corneum hydration (CM units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control diet group</td>
<td>26.4 ± 0.89</td>
<td>9.03 ± 0.11</td>
<td>2.7 ± 0.20</td>
<td>45.43 ± 2.40</td>
</tr>
<tr>
<td>Low protein diet group</td>
<td>18.4 ± 0.37*</td>
<td>8.40 ± 0.05*</td>
<td>2.0 ± 0.10*</td>
<td>32.72 ± 2.08*</td>
</tr>
</tbody>
</table>

Values are expressed means ± SE (n=6). *: P<0.05 compared with the control group.
EFFECT OF PROTEIN MALNUTRITION ON SKIN

along the crypt-villus axis [7, 32]. PM also impairs intestinal barrier function [26]. This study also showed a decrease of cell proliferative activities in the skin epidermis and stratum corneum hydration in the skin with PM. Stratum corneum hydration reflects the disruption of the skin epidermal permeability barrier function [4, 31].

Dityrosine has recently been developed as a biomarker of protein oxidation, and there have been some reports of dityrosine expression in humans [18] and experimental animals [2, 12, 20, 34, 36]. Dityrosine has been detected immunohistochemically in lipofuscin of pyramidal neurons of aged human brains [18], atherosclerotic lesions of apolipoprotein E-deficient mice [20] and cholesterol-fed rabbits [12] and renal proximal tubules in diabetic mice [34]. The dityrosine concentration significantly increased in the livers of rats chronically intoxicated with ethanol and in cooking-oil fume-induced acute lung injury in rats [2, 36]. Yet to our knowledge, this is the first report about expression of dity-
rosine in the skin epidermis of protein malnourished mice.

Witko-Sarsat et al. showed that in vivo levels of AOPPs strongly correlate with creatinine clearance, indicating that AOPPs are excellent biomarkers of the progression of chronic renal failure and uremia [35]. In the present study, the plasma BUN and creatinine concentrations and histopathological findings of the kidneys revealed that the low protein diet group did not show chronic renal failure and uremia. This result showed that increase of the plasma AOPP concentration in the low protein diet group did not affect renal condition. This study indicated that plasma AOPP concentrations were useful as biomarkers of oxidative protein damage in the skin epidermis induced by PM.

In conclusion, PM adversely affects the structure and water barrier and reservoir functions of the skin epidermis, and these pathological changes are associated with the expressions of protein oxidation markers, dityrosine and AOPPs. Though the true significance of dityrosine and AOPPs expression is unclear, these findings suggest that dityrosine and AOPPs might be useful biomarkers for estimating the degree of oxidant-mediated protein damage of the skin epidermis induced by PM.

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