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Atopic Dermatitis-like Symptoms in HR-1 Hairless Mice Fed a Diet Low in Magnesium and Zinc

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We aimed to develop an animal model for atopic dermatitis. HR-1 hairless mice fed a diet with reduced magnesium and zinc levels were compared with mice fed a standard diet. Skin dryness and wrinkle-like changes, scratching behaviour, decreased skin water content, increased transepidermal water loss and raised blood immunoglobulin E levels were seen in the group receiving the reduced magnesium and zinc diet compared with control mice. There were no significant differences in body weight or the weight of the major organs between the two groups. Haematological examination in both groups was normal apart from increased immunoglobulin E levels in mice fed a reduced magnesium and zinc diet. These mice may be useful models of atopic dermatitis; preparation of the animals is not particularly time consuming, the reproducibility is 100%, and atopic dermatitis symptoms occur even in a specific pathogen-free environment.

KEY WORDS: Atopic dermatitis; Animal model; Magnesium; Zinc

Introduction

Atopic dermatitis is a chronic disease characterized by itchy inflamed skin. There are usually periods of exacerbation and remission, and many patients have an atopic predisposition (a family and past history of bronchial asthma and allergic rhinitis, and a predisposition for the production of immunoglobulin E).[1,2] The number of patients diagnosed with atopic dermatitis has increased in recent years, but the pathology of this condition is only partly understood. Symptomatic therapy with topical steroids is the mainstay of treatment, but new therapeutic agents are required.

Animal models play a very important role in the elucidation of diseases. To date, some models have been developed for atopic dermatitis in mice,[3–6] but most have proved to be inadequate. Atopic dermatitis-like symptoms have been reported in hypomagnesaemic hairless rats[7] and low serum zinc levels observed in children with atopic eczema.[8,9] It may be possible, therefore, to create a mouse model for atopic dermatitis by feeding hairless mice a diet low in magnesium and zinc.

We aimed to develop an animal model for atopic dermatitis by feeding HR-1 hairless mice a diet containing reduced levels of

magnesium and zinc. Observations were compared with those from HR-1 hairless mice fed a standard diet.

Materials and methods

ANIMALS

Male HR-1 hairless mice, purchased from Hoshino Test Animal Breeder (Saitama, Japan), were used in all the experiments. The University Committee on Laboratory Animals gave ethical approval and the Guidelines for the Management of Laboratory Animals in Fujita Health University were followed.

FOOD

Mice were fed either standard food used for experimental mice, rats and hamsters (mouse chow CRF-1, Oriental Yeast Co. Ltd, Chiba, Japan) or standard food in which the magnesium and zinc content had been reduced by about 50%. The magnesium and zinc concentrations were 0.25 g/100 g and 6 – 7 mg/100 g, respectively, in the standard food, and 0.14 g/100 g and 3 – 4 mg/100 g, respectively, in the food with reduced mineral levels.

FEEDING PROTOCOL

Animals were kept in a specific pathogen-free (SPF) environment. After weaning at 3 weeks of age, all mice were fed normal mice food for 1 week before being divided into two groups at 4 weeks old. One group received standard food and the other group received food with reduced magnesium and zinc content. A daily amount of 4 – 6 g of food per animal was freely available.

ASSESSMENT OF SKIN

Changes in the appearance of the skin and the frequency of scratching were observed and recorded at four time points: 3, 4, 5 and 6 weeks after starting the special diet. The water content of the skin on the back of the mice, the amount of transepidermal water loss and the thickness of skin were determined 6 weeks after weaning. Water content was assessed by measuring the conductance of the skin using a Skicon 200 hygrometer (IBS Japan, Tokyo, Japan). Transepidermal water loss per unit area was determined using a Tewameter TM210 (Courage + Khazaka, Tokyo, Japan), and skin thickness was measured using a dial thickness gauge.

HISTOLOGICAL EXAMINATION OF SKIN

Rats were killed 6 weeks after the start of the controlled diets. Skin samples were taken from the eruptions, stained with haematoxylin and eosin, *O*-phenylene diamine and toluidine blue and examined histologically.

DETERMINATION OF BODY AND ORGAN WEIGHTS

In both groups body weight was determined at weekly intervals for 6 weeks after the start of feeding. When the rats were killed at 10 weeks of age the weights of the brain, heart, lung, kidneys, spleen, liver and testes were determined.

HAEMATOLOGICAL EXAMINATION

Blood samples were taken for haematological examination when the mice were 4 and 10 weeks old. Immunoglobulin E (IgE), red blood cells, white blood cells, platelet count, total protein, albumin, total cholesterol, aspartate aminotransferase, glutamate pyruvate transaminase, blood urea nitrogen and creatinine levels were measured.

STATISTICAL ANALYSIS

Results are expressed as the mean ± SD for each group. Comparisons were made using Student's *t*-test; a P-value < 0.001 was considered to be statistically significant.

Results

ANIMALS

A total of 20 male HR-1 hairless mice were used for the experiments. Ten mice received standard food, and 10 received food with a reduced magnesium and zinc content.

SKIN CHANGES

In the group receiving the reduced magnesium and zinc diet, drying and wrinkle-like changes in the skin and scratching behaviour were observed from 3 – 4 weeks after the start of feeding (Fig. 1A) compared with the standard food group (Fig. 1B). These changes became more marked with time.

On histological examination, haematoxylin and eosin staining showed thickening of the epidermis and dermis, with infiltration into the dermis of many types of cells, in the group receiving the reduced magnesium and zinc diet (Fig. 2A) compared with the standard food group (Fig. 2B). In addition, toluidine blue staining showed an increase in the mast cell count per unit area (Fig. 2C) compared with the standard food group (Fig. 2D), and *O*-phenylene diamine staining demonstrated an increase in the eosinophil count in the reduced magnesium and zinc group (data not shown).

After receiving a diet low in magnesium and zinc for 6 weeks, the water content of the skin decreased significantly compared with those receiving standard food ($P < 0.001$) (Fig. 3A). The transepidermal water loss per unit area (Fig. 3B) and skin thickness (Fig. 3C) increased significantly ($P < 0.001$ for both) in the reduced magnesium and zinc diet group compared with the standard food group.

BODY AND ORGAN WEIGHTS

No significant differences were observed with regard to body weight or weight of various organs examined at 10 weeks of age between the two diet groups (data not shown).

HAEMATOLOGICAL EXAMINATION

Blood IgE levels began to increase at about 2 – 3 weeks after the start of the reduced magnesium and zinc diet and by 10 weeks of age (that is 6 weeks after the start of feeding with the altered diet) the blood IgE level was significantly higher than in the standard food group ($P < 0.001$; Fig. 4). No other significant differences in the haematological results were found between the two groups (data not shown).

Discussion

To help elucidate the pathology of (and develop new therapies for) atopic dermatitis, a variety of murine models have been proposed.[3 – 6] Typically dermatitis is elicited in these models by repeated application of a hapten following induction of sensitization. The dermatitis produced seems to be similar to atopic dermatitis both clinically and histopathologically. The number of species of mice likely to develop the disease is limited, however, and preparing the models is time consuming.[3] It has also been reported that, in the case of the mutant NC/Nga mouse model, dermatitis is induced by parasitic mites; atopic dermatitis is therefore not induced under SPF conditions, and the reproducibility is poor.[4] By feeding HR-1 hairless mice a reduced magnesium and zinc diet we produced a murine model with characteristics observed in patients with atopic dermatitis. These characteristics include skin dryness, scratching behaviour, decreased skin water content, increased transepidermal water loss and raised blood IgE levels. This is a potentially useful model since preparation of the mice is not particularly time consuming, the

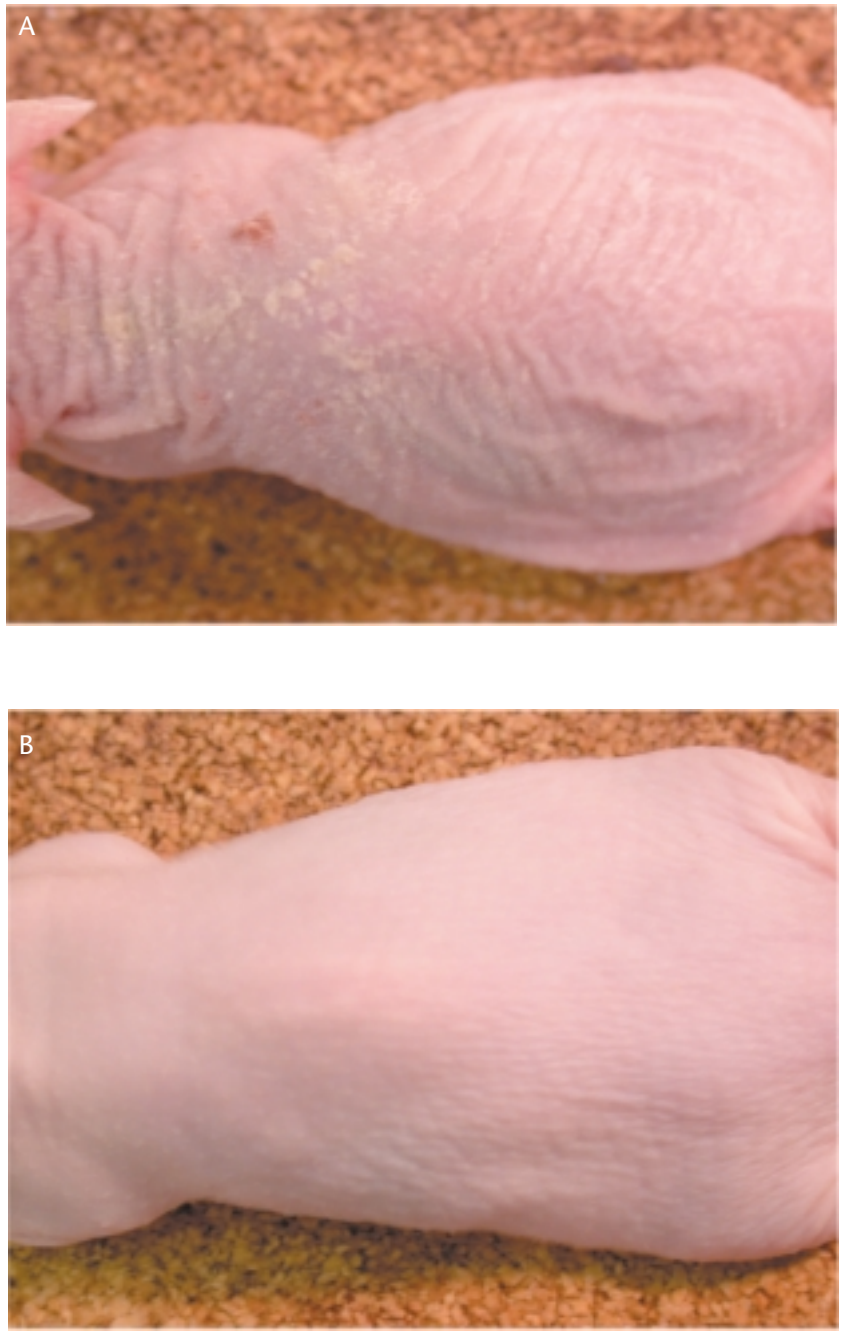

FIGURE 1: Macroscopic findings after 6 weeks in mice fed a diet with a reduced magnesium and zinc content (A) or a standard diet (B). Drying and wrinkle-like changes in the skin and scratching behaviour were observed in the group receiving the reduced magnesium and zinc diet

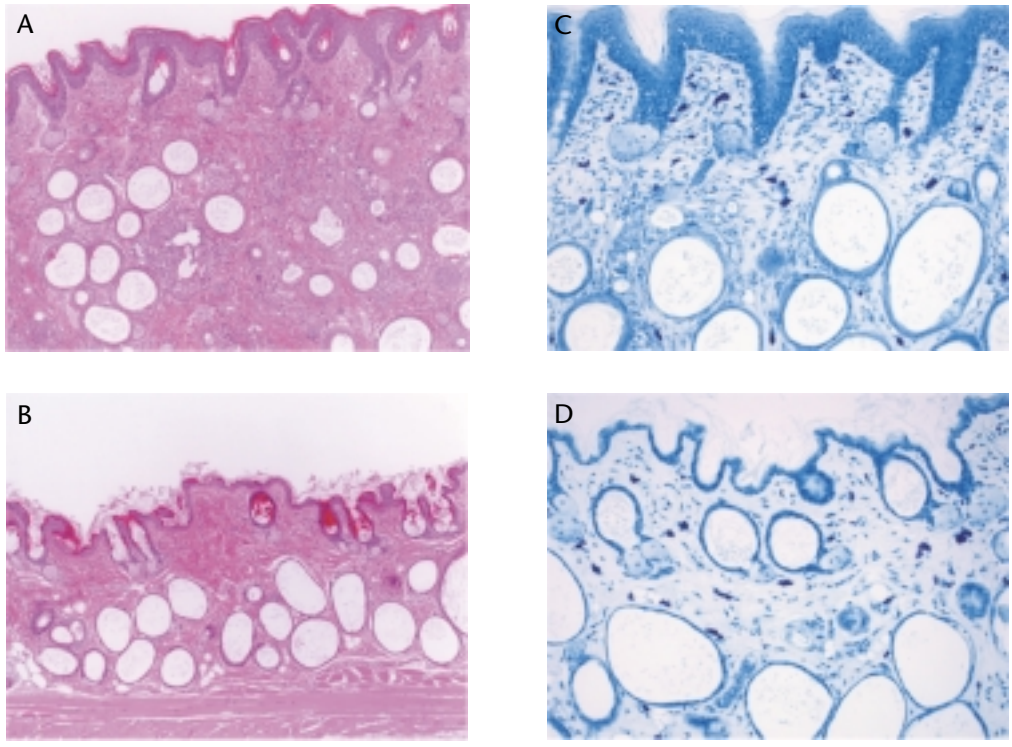

FIGURE 2: Histological findings after 6 weeks in mice fed a diet with a reduced magnesium and zinc content (A and C, × 100) or a standard diet (B and D, × 200). There was thickening of the epidermis and dermis, and infiltration of many types of cells into the dermis in the group receiving food with a reduced magnesium and zinc content (A) compared with the standard food group (B; haematoxylin and eosin staining). There was also an increase in the mast cell count per unit area in the group receiving the reduced magnesium and zinc diet (C) compared with the standard food group (D; toluidine blue staining)

reproducibility is 100%, and the animals develop similar symptoms in an SPF environment as in a conventional environment (unpublished observations).

Immunohistological examination of the skin from mice fed the reduced magnesium and zinc diet showed significant increases in CD4-positive T-cells, IgE, interleukin 4 and interleukin 5 in the dermis compared with mice fed standard food (unpublished observations). Further studies have also demonstrated that the pruritus produced in mice receiving a diet low in magnesium and zinc is inhibited by antihistamine drugs (unpublished observations).

The development of dermatitis has been reported in rats with magnesium deficiency.[7] In addition, it is known that zinc deficiency is associated with refractory eruption and poor healing of skin wounds. Furthermore, a decrease in the serum zinc concentration has been reported in patients with atopic dermatitis.[8,9] We initially fed HR-1 hairless mice a diet in which only zinc levels were reduced. A slight dryness of the skin was observed, but rapid weight loss also occurred

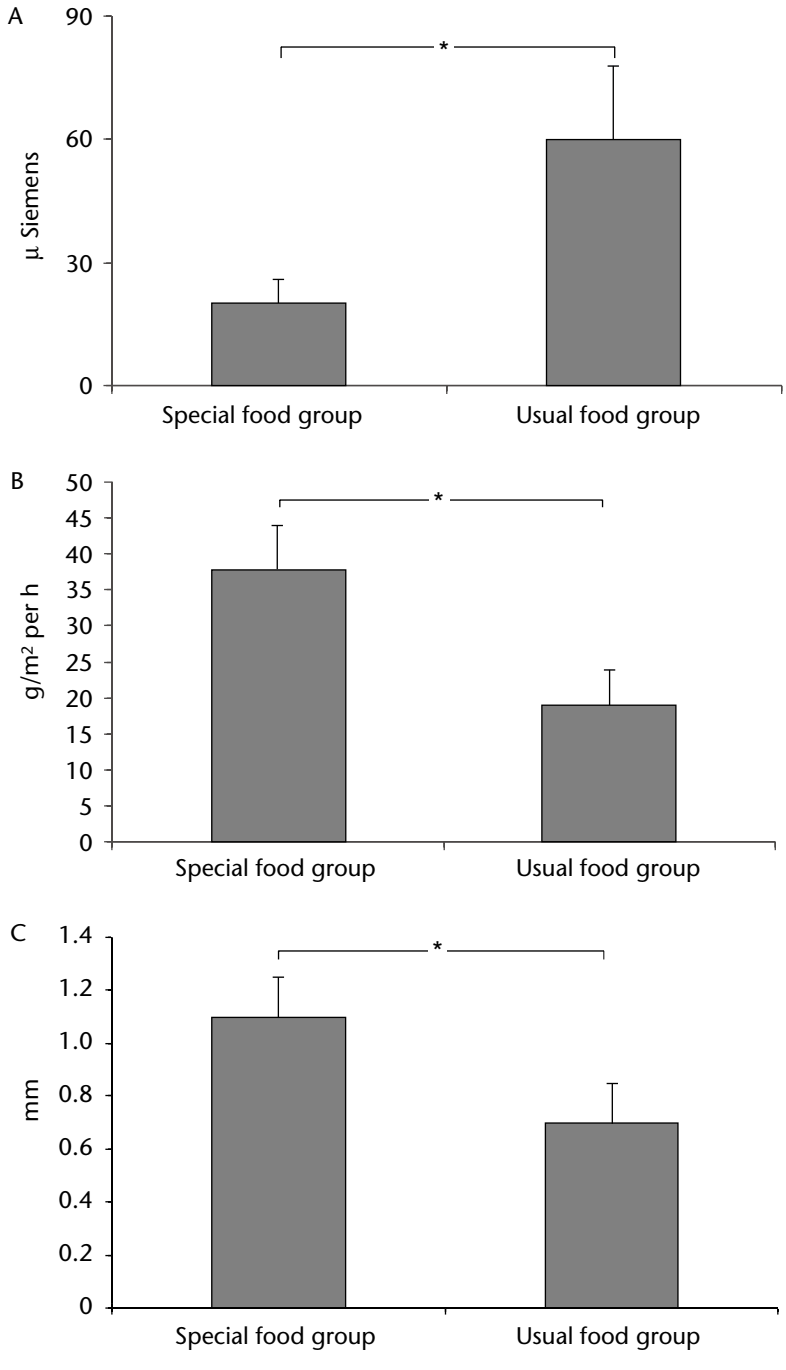

FIGURE 3: Comparison of skin water content (A), transepidermal water loss (B) and skin thickness (C) between mice fed a reduced magnesium and zinc diet and mice fed a standard diet. Values given are the mean ± SD. *$P < 0.001$ using Student's *t*-test

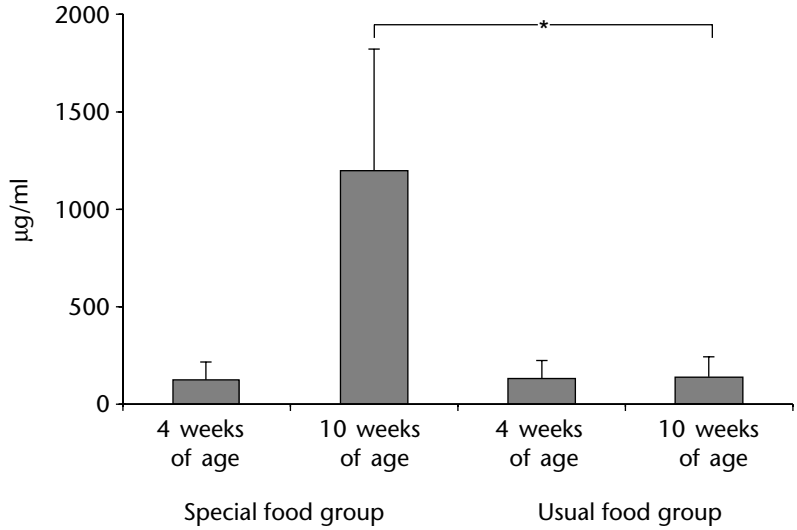

FIGURE 4: Comparison of blood immunoglobulin E levels at 4 and 10 weeks of age between 10 mice fed a reduced magnesium and zinc diet (special food group) and 10 mice fed a standard diet (usual food group). Values given are the mean ± SD. *$P < 0.001$ using Student's *t*-test

and the prognosis was poor. By adjusting the amounts of both magnesium and zinc, we succeeded in producing a murine model for atopic dermatitis. This effect, however, seems to be specific for HR-1 hairless mice: no atopic dermatitis-like symptoms were produced when Balb/c, C57BL and ICR mice were fed a diet with reduced concentrations of magnesium and zinc (unpublished observations). Thus a genetic abnormality may exist in HR-1 hairless mice that is required for the appearance of the observed atopic dermatitis-like symptoms. Further studies are needed to elucidate this possibility.

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