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ORIGINAL ARTICLE

Effect of cyclodextrin on allergic action of the PiCl-induced in NC/Nga mice

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Abstract Itching dermatitis, which is very similar to human atopic dermatitis, arises spontaneously in NC/Nga mice. Changes in lesional skin and the plasma concentration of IgE during the development of atopic dermatitis-like disease up to 8 weeks after the start of picryl chloride (PiCl) in NC/Nga mice were examined. In the mice which atopic dermatitis had been induced, 28-day administration of α cyclodextrin (α CD) significantly inhibited aggravation of detmatitis without having effects on body weight. Clinical signs and symptoms seen in PiCl treated NC/Nga mice began with erythema and haemorrhage, following oedema, superficial erosion, deep excoriation, scaling and dryness of the skin. An increase was observed in the number of mast cells and eosinophil infiltration in the lesional skin. Almost human patients with nasal inflammation or bronchial asthmatics symptoms disappeared by oral administration of α CD (5 mg/day) for 2 month. These results suggest that α CD is useful for treatment of human atopic dermatitis.

Keywords α Cyclodextrin · Allergic action · Atopic dermatitis · IgE · NC/Nga mice

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Introduction

It is well known that itching dermatitis, which is very similar to human atopic dermatitis, arises spontaneously in NC/Nga mice. The conventional NC/Nga mice were useful as a model of human atopic dermatitis. [1–3] The mice raised in conventional (non-sterile) circumstances spontaneously develop human atopic dermatitis-like skin lesions with hyper IgE production, while the ones raised in specific pathogen-free environment show neither dermatitis nor hyper IgE production. In this study, NC/Nga mice were feed either standard feed with or without α CD and glycomannam for 8 weeks under specific pathogen free conditions. The NC/Nga mouse thus provides a good animal model for evaluating potential therapeutic drugs for human atopic dermatitis. The elevation plasma IgE level in the plasma gradually increases to very high levels, and reaches a peak at the age of 16–18 weeks. Further the plasma IgE level in the plasma has been reported to correlate with the appearance skin lesions in NC/Nga mice [4].

We examined dermatitis observation, IgE level in plasma during the development of atopic dermatitis-like disease up to 8 weeks after the start of 2,4,6-Trinitro chlorobenzene (PiCl) induction in NC/Nga mice.

Materials and methods

Animals

Male NC/Nga mice aged 6 weeks bred under specific pathogen-free (SPF) condition were purchased from Shimizu Animal Laboratory Co., Japan. The mice were

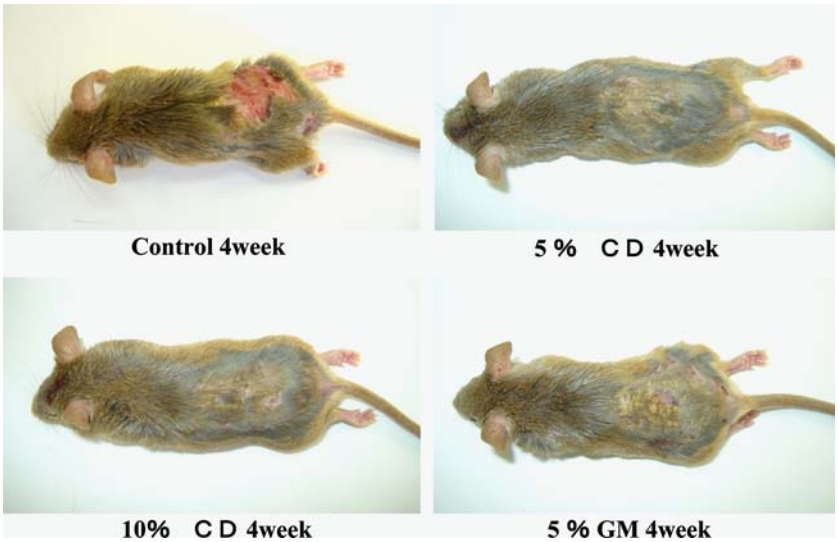

Fig. 1 Appearance of NC/Nga mice at 4 weeks after induction of PiCl

given γ-ray irradiated food with or without α CD and glycomannam (GM). Five mice were used for skin observation, histological examination and biochemical examination. The mice were kept in an air-controlled barrier system environment during the experimental period.

PiCl was purchased from Tokyo Kasei Chemical Co., Japan. A mouse IgE EIA kit was purchased from YAMASA Corp., (Tokyo, Japan).

Sensitization and induction

The furs of the thoracic and abdominal regions under anaesthetized animals were shaved off with a hair-clipper 1 week before the sensitization. Using a micropipette, 150 μl of the sensitizing 5% PiCl solution (PiCl dissolved in solvent consisting a mixture four parts ethanol to one parts acetone) was applied to the thoracic and abdominal areas.

Four days after the sensitization, induction was performed. A micropipette was used to apply 200 μl of PiCl solution (0.8% PiCl olive oil/ethanol solution) to thoracic and abdominal areas under ether-anaesthetized animals. The procedure was repeated once a week for up to 8 weeks.

Dermatitis observation (scored evaluation)

Evaluation items were scored as following: 0 = no sign; 1 = mild; 2 = moderate; 3 = severe, 1,2,4 and 8 weeks after the start of induction treatment based on the clinical evaluation standards was used to evaluate atopic dermatitis-system. The observation items were (1) flare and the haemorrhage, (2) oedema, (3) excoriation and erosion, and (4) incrustation and xerosis except scratching behavior. The sum of the scores for each evaluation items (maximum score 12) was taken as the dermatitis score.

Histopathological analysis

The pieces of skin from dorsal were fixed with 10% buffered formalin solution (Wako Pure Chemical Industries, Ltd, Japan), embedded in paraffin by the conventional method and cut into 3–4 μm sections. Serial sections were stained with hematoxylin-eosin using Mayer's Haematoxylin Solution (Wako Pure Chemical Industries, Ltd, Japan) and toluidine blue using 0.05% Toluidine Blue Solution (Muto Pure Chemicals, Japan) to identify mast cells.

Measurement of plasma level of IgE

Blood was withdrawn from jugular vein of ether anaesthetized NC/Nga mice 0, 1, 2, 4 and 8 weeks after the start of the induction treatment. Plasma samples were obtained by centrifugation. Total IgE levels were

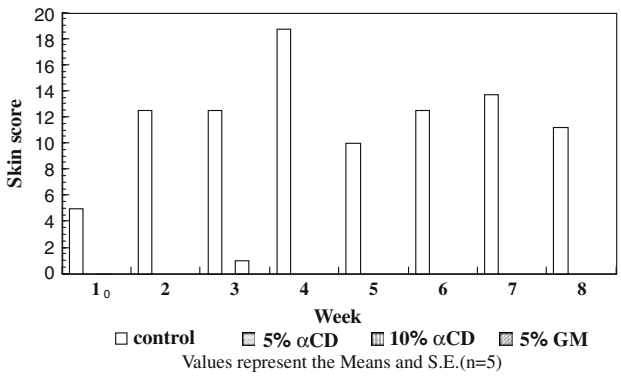

Fig. 2 Effect of α CD on skin score of the PiCl-induced allergic dermatitis in NC/Nga mice

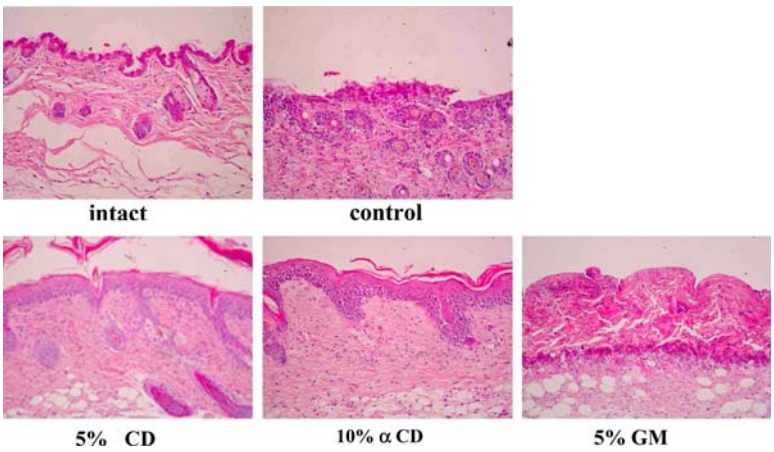

Fig. 3 Sections after 4 weeks derived from NC/Nga mice

measured with sandwich mouse IgE ELISA (Yamasa Corp., Japan).

Data analysis and statistics

The IgE levels in plasma were expressed as the ± standard error of the mean (SEM), and were analyzed using the Wicoxon rank sum the test. $^*P < 0.05$ was considered significant.

Results and discussion

Development of AD-like skin lesions

Figure 1 shows gross findings of NC/Nga mice after 4 week PiCl induced. Atopic dermatitis–like lesions could find on the face, neck and dorsal skin of the control mice. These symptoms were observed clinically on the face area, neck and dorsal skin in the control mice.

Figure 2 shows the atopic dermatitis score of skin symptoms up to 8 weeks. The atopic dermatitis score gradually increased with time until 6 weeks. Dermatitis was noted from 1 week after the start of the induction treatment except 10% α CD. Clinical signs and symptoms can be seen in PiCl-treated by edema, superficial erosion, deep excoriation, scaling and dryness of the skin as well as retarded growth, and the changes were exacerbated with an increase in the number of 4 weeks induction of PiCl application. However 5% and 10% α CD suppressed the increase of the dermatitis score compared to the control mice at 8 weeks.

Histopathological study

In order to clarify the efficacy of α CD histochemically, paraffin embedded dorosal sections, where dermatitis developed in NC/Nga mice, were stained hematoxylin-eosin (Fig. 3) and with toluidine-blue (Fig. 4). Hematoxylin-eosin staining is widely used hematoxylin stains

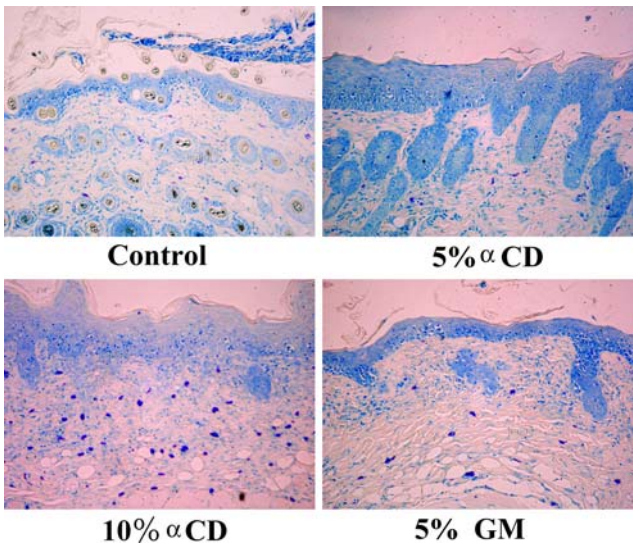

Fig. 4 Microscopic of toluidine blue-stained the dorsal sections after 4 weeks derived from NC/Nga mice

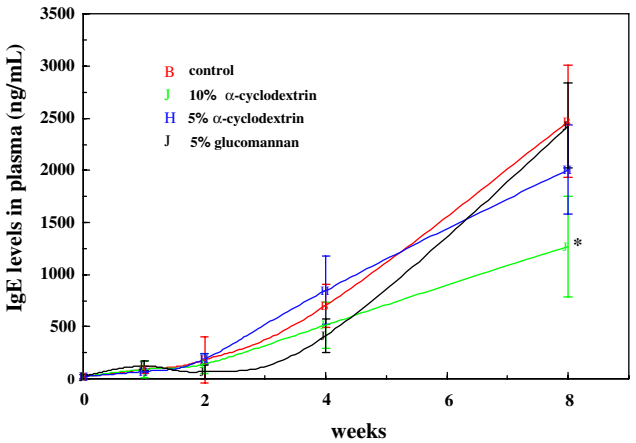

Fig. 5 Effect of α CD on plasma level of IgE in NC/Nga mice

	Nasal inflammation symptom		
Sex / Age	disappearance	mitigation	no effect
F / 31	○		
F / 43	○		
F / 44	○		
M / 46	○		
M / 55	○		
M / 38		○	
F / 46		○	
F / 57		○	
M / 33			○
M / 36			○

	Bronchial asthmatics symptom		
Sex / Age	disappearanc	mitigation	no effect
M / 11	○		
M / 41	○		
M / 47	○		
F / 54	○		

α CD was administarated 5g/day for 2 month.

Fig. 6 Effect of α CD on Nasal inflammation symptom and brochial asthmatics symptom of patients

nuclei violet, and eosin stains fiver, interstitial tissue and cytosol pink. On histopathological examination, the severity of the skin lesions as by hyperkeratosis (thicking of the staratum corneum in the epidermis) and acanthosis (thickening of the staratum spinosum in epidermis) and dermatitis also gradually increased from 2 weeks after the start of induction treatment.

Toluidine-blue stains the granules of granulocytes red to violet. There were marked histopathlogical changes, such as infiltration of granuocytes into the epidermal and of mast cells (Fig. 4) into the dermis and the subcutaneous tissue, as compared to control mice. Degranulation of granulocytes was observed in the skin of control group, but was suppressed in 10% α CD group.

Plasma levels of IgE

Plasma IgE Levels of at 0 (intact), 1, 2, 4 and 8 weeks were measured after the start of the induction treatment. (Fig. 5) The elevation of IgE level has been reported to correlate with the appearance of skin lesions in NC/Nga mice. [4] The level of IgE in the plasma gradually increased to a very high level, and it reached a peak at the age of 16–18 weeks. There were no differences in total plasma IgE levels between intact and PiCl treated mice at 1 and 2 weeks. However, the levels at 4 and 8 weeks were higher than those of intact animals. 10% α CD was significantly suppressed the IgE level than that under the control at 8 weeks.

The elevation of IgE level is due to T helper 2 differentiation [3]. Consistent with the previous report [5], the plasma IgE level was gradually increased by repeated challenges by PiCl treatment. This elevation of IgE level was significantly suppressed by oral dose of α CD, indicating its ability to downregulate the T helper 2 response.

Patients suffering from atomic dermatitis, especially adults with severe symptoms such as nasal inflammation and bronchial asthmatics, are increasing in Japan. Elevation of serum IgE levels is also a characteristic feature in many patients [6, 7]. Symptoms of patients with nasal inflammation (8 in 10 person) and bronchial asthmatics symptoms (4 in 4 person) disappeared by oral administration of α CD (5 g/day) for 2 months. (Fig. 6) The results of the present study show the oral administration of a CD can prevent a part of development of atopic dermatitis in NC/Nga mice and human atopic dermatitis. α CD may be suppressed the degranulation of granulocytes (Fig. 4) by inhibition IgE production. (Fig. 5) Therefore it is suggested that α CD is useful to suppress human atopic dermatitis.

In conclusion, α CD has been successfully applied in treating atopic dermatitis induced with PiCl in NC/Nga mice and human atopic dermatitis, inhibition in the dermatitis and inflammatory in the lesions. Furthermore, the mice antigen-induced allergic dermatitis model in NC/Nga mice are seem to be useful for the basic study of atopic dermatitis.

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