A Novel Immunomodulator, FTY720, Prevents Spontaneous Dermatitis in NC/Nga Mice

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Oral administration of a novel immunomodulator, FTY720 (0.1 mg/kg, once a week), completely prevented the spontaneous development of dermatitis in NC/Nga mice. It also strongly suppressed hyper IgE production in serum, as well as hypertrophy of the epidermis and the degranulation of granulocytes in the skin, all of which were observed in mice in which the dermatitis had become established. These results strongly suggest that FTY720 is a promising candidate for treatment of human atopic dermatitis.

Key words FTY720; atopic dermatitis; NC/Nga mouse

The novel immunomodulator (immunosuppressant) FTY720 is a synthetic structural analogue of myriocin (ISP-1), a metabolite of Isaria cinclarii. FTY720 was discovered by Tetsuro Fujita, in collaboration with Taito Co., Ltd., Japan and Yoshitomi Pharmaceutical Industries, Ltd., Japan. FTY is an abbreviation of Fujita, Taito and Yoshitomi. The efficacy of FTY720 has been well established in preclinical transplantation models (rat heart, liver, skin, small intestine, dog kidney and monkey kidney), and also recently in renal transplantation in humans. The mechanism of action of FTY720 differs from that of established immunosuppressants (cyclosporin and tacrolimus hydrate). FTY720 is phosphorylated by sphingosine kinase, and the product, FTY720 monophosphate, is the active form of the drug. FTY720 monophosphate acts as a potent agonist at four sphingosine 1-phosphate receptors (S1P), especially S1P2 (endothelial differentiation gene (edg) 6) and S1P5 (edg-8), and modulates chemotactic responses of lymphocytes and lymphocyte trafficking. A result, FTY720 suppresses immune response by sequestering lymphocytes from blood and peripheral tissues to the secondary lymphoid tissues. However, Sugito and Fukuzawa reported that administration of FTY720 to mice without lymph nodes, Payer’s patches and lymph nodes, still resulted in peripheral lymphopenia, prevented the infiltration of CD4+ T cells into skin allografts, and prolonged skin allograft survival. Even though the mechanisms of pharmacological action of FTY720 remain to be fully clarified, it is clear that FTY720 has no inhibitory effect on cytokine production, in contrast to the established immunosuppressants.

NC/Nga mice have been used as an animal model for human atopic dermatitis. The mice raised in conventional (nonsterile) circumstances spontaneously develop human atopic dermatitis-like skin lesions with hyper IgE production, while those raised in a specific pathogen-free environment show neither dermatitis nor hyper IgE production.

In this study, we examined the efficacy of FTY720 for preventing the development of dermatitis in this animal model (NC/Nga mice) of human atopic dermatitis.

MATERIALS AND METHODS

Animals NC/Nga mice (4-week-old males) bred under specific pathogen-free (SPF) conditions were purchased from Japan SLC Inc., Shizuoka, Japan. The mice were given γ-ray-irradiated food (CRF-1, Oriental Bio Co., Kyoto, Japan) and distilled water for injection (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan).

FTY720 2-Amino-2-[2-(4-octylphenyl)ethyl]propane-1,3-diol hydrochloride (FTY720) was given kindly from Yoshitomi Pharmaceutical Industries, Ltd., Japan.

Study Protocol Twelve NC/Nga mice were divided into three groups (n=4/group), of which two (treated and untreated groups) were kept under conventional conditions, and one (control group) was kept in an SPF environment. From 5 weeks of age (when the mice had no skin lesions), the treated group was orally given FTY720 in water (0.1 mg/kg) once a week. The untreated and the control groups were given the vehicle (water) alone. The development of dermatitis was observed at weekly intervals. At 25 weeks of age, skin from the buttocks and shoulders (where dermatitis had developed in the untreated group), lymph nodes, and spleen were excised under sodium pentobarbital anesthesia.

Histochemical Staining Pieces of skin from the shoulder were fixed with 10% buffered formalin solution (Wako Pure Chemical Industries, Ltd., Osaka, Japan), embedded in paraffin by the conventional method and cut into 3 to 4 μm sections. The sections were stained with hematoxylin-eosin using Mayer’s Hematoxylin Soln. (Wako Pure Chemical Industries, Ltd.) and sodium tetrabromofluorescein (Wako Pure Chemical Industries, Ltd.), and with toluidine blue using 0.05% Toluidine Blue Solution (Muto Pure Chemicals Co., Ltd., Tokyo, Japan).

Measurement of Serum IgE Level. (Anti-mouse IgE e-Chain) IgG-Coated Polystyrene Balls Polystyrene balls (3.2 mm in diameter, Immuno Chemical Inc., Okayama, Japan) were coated with sheep (anti-mouse IgE e-chain) IgG (Calbiochem-Novabiochem Corporation, La Jolla, CA, U.S.A.) by physical adsorption.

(Anti-mouse IgE e-Chain) Fab’-Horseradish Peroxidase Conjugate Sheep (anti-mouse IgE e-chain) IgG was digested with papain to afford F(ab’)_2. F(ab’)_2 was reduced...
to Fab’ by incubation with 2-mercaptoethylamine, and Fab’ was conjugated with horseradish peroxidase using N-succinimidyl-6-maleimidohexanoate (Dojindo Laboratories, Inc., Kumamoto, Japan).

Measurement of Serum IgE Level Serum samples were diluted 1000-fold with 10 mM sodium phosphate buffer, pH 7.0, containing 0.1 M NaCl, 1 g/l bovine serum albumin (BSA, Fraction V, Intergen Co., Purchase, NY, U.S.A.) and 1 g/l NaN₃. The diluted serum (0.1 ml) or standard IgE (monoclonal mouse (anti-DNP) IgE, Seikagaku Co. Tokyo, Japan) was used as a standard in the same buffer (0.1 ml) was mixed with 10 mM sodium phosphate buffer, pH 7.0, containing 1.0 M NaCl, 1 g/l BSA and 1 g/l NaN₃ (0.05 ml). The mixture was incubated with a sheep (anti-mouse IgE ε-chain) IgG-coated polystyrene ball at 37 °C for 3 h and 4 °C overnight. After incubation, the reaction mixture was removed by aspiration, and the polystyrene ball was washed twice with 2 ml of ice-cold 10 mM sodium phosphate buffer, pH 7.0, containing 0.1 M NaCl. The polystyrene ball was incubated with sheep (anti-mouse IgE ε-chain) Fab’-horseradish peroxidase conjugate (5 ng) in 10 mM sodium phosphate buffer, pH 7.0, containing 0.1 M NaCl and 1 g/l BSA (0.15 ml) at 20 °C for 3 h. After incubation, the conjugate solution was removed by aspiration, and the polystyrene ball was washed as above. Finally, peroxidase activity bound to the polystyrene ball was measured by fluorometry by using p-hydroxyphenylpropionic acid as a hydrogen donor.

Statistical Analysis The significance of differences in serum IgE level was evaluated by using the Mann–Whitney U-test.

RESULTS

Effect of FTY720 on the Development of Dermatitis NC/Nga mice, which spontaneously develop human atopic dermatitis-like skin lesions under conventional (nonsterile) conditions, but not in a specific pathogen-free (SPF) environment, were used to evaluate the efficacy of FTY720 for preventing the development of dermatitis. Mice (n=12, male) with no skin symptoms were divided into the following three groups (n=4/group), 1) the treated group: kept in a conventional environment and given FTY720 in water (0.1 mg/kg, once a week by oral administration), 2) the untreated group: kept in a conventional environment and given the vehicle (water) alone, and 3) the control group, kept in an SPF environment and given the vehicle (water) alone.
Fig. 3. Effect of Oral Administration of FTY720 on Hypertrophy of the Epidermis in Skin at 25 Weeks of Age

A piece of skin from the shoulder was fixed with 10% buffered formalin solution, embedded in paraffin by the conventional method, and cut in 3 to 4 μm sections. The sections were stained with hematoxylin–eosin. Upper small boxes are ×1000, and lower large boxes are ×400. Alignment of photographs is the same as in Fig. 1.

Fig. 4. Effect of Oral Administration of FTY720 on Degranulation of Granulocytes in Skin at 25 Weeks of Age

A piece of skin from the shoulder was fixed with 10% buffered formalin solution, embedded in paraffin by the conventional method, and cut into 3 to 4 μm sections. The sections were stained with toluidine blue. Upper small boxes are ×1000, and lower large boxes are ×400. Alignment of photographs is the same as in Fig. 1.
ventitional environment and given the vehicle (water) alone, and 3) the control group: kept in an SPF environment and given the vehicle (water) alone. Photographs of every mouse at 22 weeks of age are shown in Fig. 1. In the untreated group, all mice developed dermatitis at the shoulders and buttocks by 19 weeks of age, while no skin lesion was observed in the control group (Figs. 1, 2). Matsuda et al. reported that dermatitis appeared on the face, neck, ears and dorsal skin of the mice, with onset at 7 to 8 weeks of age. The differences in the sites involved and the age of appearance may be explained by environmental differences between our and their breeding rooms. In the treated group, the development of the dermatitis was completely suppressed, i.e., no skin lesion was observed up to 25 weeks of age (Figs. 1, 2). These results clearly show that FTY720 can prevent the development of dermatitis in NC/Nga mouse.

**Histochemical Studies** To clarify the efficacy of FTY720 histochemically, paraffin-embedded skin sections from the shoulder, where dermatitis had developed in the untreated group, were stained with hematoxylin–eosin (Fig. 3) and with toluidine blue (Fig. 4). Hematoxylin–eosin staining is widely used; hematoxylin stains nuclei violet, and eosin stains fiver, interstitial tissue and cytosol pink. Epidermis (shown in the small boxes in Fig. 3) showed extensive hypertrophy in the untreated group (Nos. 5—8) in comparison with the control group (Nos. 9—12), and this hypertrophy was suppressed in the treated group (Nos. 1—4) (Fig. 3). Toluidine-blue staining the granules of granulocytes red to violet. Degranulation of granulocytes, as shown in the small boxes in Fig. 4, was observed in the skin in the untreated group (Nos. 5—8), but was suppressed in the treated group (Nos. 1—4) (Fig. 4).

**IgE Level in Peripheral Blood** To clarify the efficacy of FTY720 immunologically, the IgE concentration in serum was measured by means of a two-site enzyme immunoassay. At the period when skin lesions appeared, hyper IgE production was observed in the untreated group. No elevation of IgE concentration was observed in the treated or control group (Fig. 5).

**DISCUSSION**

The increase in the number of patients with atopic dermatitis has led to a requirement for new means to prevent the onset and to treat the disease. Tacrolimus hydrate (FK506) ointment has recently been approved for this purpose. The novel agent FTY720 has a number of attractive properties. 1) The proposed mechanism of immunosuppressive effect of FTY720 is different from that of established drugs such as glucocorticoids, immunosuppressive drugs (tacrolimus, cyclosporin) and anti-allergic drugs (ketotifen fumarate, fexofenadine hydrochloride, pranlukast hydrate, suplatast tosilate, and so on). 2) At therapeutic doses, FTY720 does not affect T cell and B cell responses in vitro, while in vivo, no increase in susceptibility to infectious diseases was observed and the immune memory function was not impaired. 3) No critical adverse effect was observed at therapeutic doses in toxicity tests.

The results of the present study show that oral administration of FTY720 can completely prevent the development of dermatitis in NC/Nga mice. FTY720 suppressed the degranulation of granulocytes (Fig. 4) by suppressing hyper IgE production (Fig. 5). To examine the mechanisms involved, mRNA levels of Th1 cytokines (interleukin-2 and interferon-γ) and Th2 cytokines (interleukin-4 and interleukin-5) in the peripheral blood cells (at 19—20 weeks and 25 weeks of age), lymphocytes from lymph nodes (25 weeks of age), and spleen cells (25 weeks of age) were compared among the three groups by RT-PCR. However, we found no apparent correlation of the cytokine mRNA levels with the drug efficacy (data not shown). Thus, further study to determine whether FTY720 affects the activation, differentiation and chemotaxis of granulocytes and monocytes, antigen presentation by dendritic cells, and so on are needed to determine how the hyper IgE production prevents.

In conclusion, the results of this study strongly suggest that FTY720 is a good candidate drug for the prevention of human atopic dermatitis. Although the efficacy of FTY720 to improve established dermatitis remains to be defined, FTY720 should at least be useful to prevent the recurrence of atopic dermatitis after treatment with glucocorticoid ointment and/or tacrolimus hydrate ointment.

**REFERENCES**


