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Original article

Therapeutic approach to mite-induced intractable dermatitis using novel immunomodulator FTY720 ointment (fingolimod) in NC/Nga mice

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Abbreviations:
AD, atopic dermatitis; Df, *Dermatophagoides farinae* crude extract; Ig, immunoglobulin; S1P, sphingosine 1-phosphate; SC, stratum corneum; TEWL, transepidermal water loss; TJ, tight junction

ABSTRACT

Background: The increasing incidence and prevalence of atopic dermatitis (AD) demands new therapeutic approaches for treating the disease. We investigated the therapeutic efficacy of immunomodulator FTY720 ointment (fingolimod) for mite-induced intractable AD using an NC/Nga mouse model.
Methods: Female NC/Nga mice that developed severe AD were divided into four groups: (1) FTY720 (0.001% FTY720 ointment), (2) tacrolimus (tacrolimus hydrate ointment) (3) betamethasone (betamethasone ointment), and (4) ointment base (hydrophilic petrolatum), all of which received treatment six times per week. Therapeutic efficacy after two weeks was evaluated in terms of AD severity, histochemical observations (epidermal hypertrophy, mast cell accumulation, and $CD3^+$ T cell infiltration), transepidermal water loss (TEWL), and epidermal barrier function (filaggrin expression).
Results: Betamethasone treatment showed little effect, confirming that the AD was intractable. In the FTY720 group, AD improved significantly compared with the ointment base group, as did epidermal hypertrophy, mast cell accumulation, and $CD3^+$ T cell infiltration. In contrast, AD in the tacrolimus and betamethasone groups did not improve significantly, nor did epidermal hypertrophy or mast cell accumulation. Furthermore, in the FTY720 group, TEWL decreased significantly compared with the ointment base group, and filaggrin expression significantly increased compared with the betamethasone and ointment base groups.
Conclusions: FTY720 ointment is a promising candidate for treatment of intractable AD. These findings also provide the first evidence that FTY720 ointment ameliorates epidermal barrier function.

Introduction

Atopic dermatitis (AD) is the most common skin disease and can significantly compromise quality of life due to sleep disruption, social awkwardness, and emotional distress. Most infants who present with mild AD will outgrow their skin disease in later childhood.[1] Recent studies have indicated that defects in epidermal barrier function (tight junction [TJ] and stratum corneum [SC] barriers) contribute greatly to triggering and perpetuating skin inflammation associated with AD. With AD, the skin is characterized by increased transepidermal water loss and reduced levels of ceramides and filaggrin.[1] Claudin-1 and filaggrin play a critical role in TJ and SC barrier formation, respectively. In AD, therapeutic targets include not only the inflammatory response but also dry skin associated with epidermal barrier dysfunction.

The novel immunomodulator FTY720 (fingolimod) was synthesized by structural modification of myriocin (ISP-I), a compound from *Isaria sinclairii*.[2,3] FTY720 was discovered by Tetsuro Fujita (F) in collaboration with Taito Co., Ltd. (T; Mitsui Sugar, Tokyo, Japan) and Yoshitomi Pharmaceutical Industries, Ltd. (Y; Mitsubishi Tanabe Pharma Corporation, Osaka, Japan) in Japan. As a result of structural modification studies of ISP-I, the reduction of toxicity and the enhancement of immunosuppressive activity were acquired. FTY720 has been reported to be effective not only in preclinical transplantation models, but also in preventing development of various immunologic diseases in animal models, including rheumatoid arthritis,[4] myasthenia gravis,[5] multiple sclerosis,[6] type 1

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diabetes mellitus,[7,8] and AD.[9–11] FTY720 was approved for treatment of human multiple sclerosis in the Russia in 2010. Thereafter, it has been approved for use in at least 50 countries (United States, European Union, Japan, etc.).

The mechanism of action of FTY720 differs from that of established immunosuppressants, such as tacrolimus hydrate and cyclosporine. *In vivo,* FTY720 is rapidly phosphorylated by sphingosine kinase 2 to phospho-FTY720 — the active form of the drug. Phospho-FTY720 is an agonist of sphingosine 1-phosphate receptor [S1PR]. Interestingly, phospho-FTY720 is able to bind to four S1PRs ($S1P_1$, $S1P_3$, $S1P_4$, and $S1P_5$), rendering it a potentially useful agonist of S1PR. This signaling induces internalization and intracellular partial degradation of the receptor.[12–14] As a result, FTY720 suppresses the immune response by sequestering circulating mature lymphocytes from the blood and peripheral tissue to the secondary lymphoid tissue and thymus.[15,16] In contrast, $S1P_2$ binds S1P but not phospho-FTY720. At oral therapeutic doses, FTY720 does not affect T cell and B cell responses *in vitro* or *in vivo*.[9,17] Because FTY720 treatment allows for preservation of many aspects of immune function, including the total number of lymphocytes, capacity for lymphocyte activation in the lymph nodes and tissue, capacity for generating antibodies, and innate immune response,[18,19] there is only a limited increase in susceptibility to infectious disease, such as herpes virus infection and urinary tract infection.[20] Furthermore, immune memory function is not impaired.[17]

NC/Nga mice have been used as a murine model of human AD.[21] In conventional circumstances, the human AD-like skin lesions spontaneously appear with hyper-immunoglobulin E (IgE) production, while in a specific pathogen-free environment, mice show neither AD nor hyper-IgE production.[21] We previously reported that oral FTY720 in combination with betamethasone ointment significantly improved spontaneous[10] and mite-induced[11] AD in an NC/Nga mouse model.

In the present study, we examined the local efficacy of FTY720 ointment for treating established steroid-resistant (intractable) AD using an NC/Nga mouse model.

Methods

Animals and ethics

Nine-week-old female NC/Nga mice bred under specific pathogen-free (SPF) conditions were purchased from Japan SLC Inc., Shizuoka, Japan. The mice were bred and maintained under SPF conditions (23 ± 1 °C and 47–67% humidity, under a 12 h light/dark cycle), and given γ-ray-irradiated food (RCF-1; Orientalbio Co., Ltd., Kyoto, Japan) and distilled water ad libitum. This study was performed according to a protocol approved by the Institutional Animal Care Committee of Setsunan University (Nos. 12-11-16-02-S-239 and 12-12-16-02-S-278). Throughout the experimental procedures, every effort was made to minimize animal suffering and the number of animals used.

Drugs

2-amino-2-[2-(4-octylphenyl)ethyl]propane-1,3-diol hydrochloride (FTY720, fingolimod) was kindly provided by Yoshitomi Pharmaceutical Industries, Ltd., Japan. 0.001% FTY720 ointment was prepared by mixing 0.5 mL of 0.1 mg/mL FTY720 with 4.5 g of hydrophilic petrolatum. Betamethasone valerate ointment (0.12%

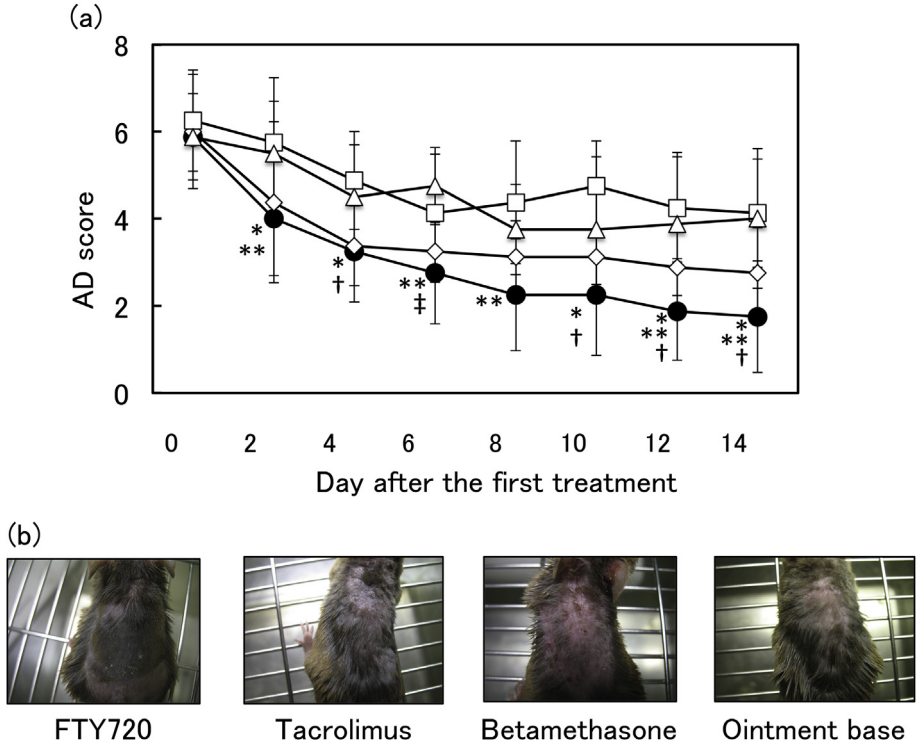

Fig. 1. Therapeutic effects of each treatment on atopic dermatitis in NC/Nga mice. Female NC/Nga mice with severe skin lesions were treated for two weeks with (1) FTY720 (0.001% FTY720 ointment, 100 mg/affected area, n = 8, ●), (2) tacrolimus (tacrolimus hydrate ointment, 100 mg/affected area, n = 8, ◇), (3) betamethasone (betamethasone ointment, 100 mg/affected area, n = 8, □), or (4) ointment base (hydrophilic petrolatum, 100 mg/affected area, n = 8, △) six times per week. **(a)** Each value is the mean, while the vertical bar with small horizontal bars indicates the standard deviation. The significance of the difference in atopic dermatitis (AD) score was examined using the Mann–Whitney *U* test. *(the FTY720 group *vs.* betamethasone group), **(the FTY720 group *vs.* the ointment base group), †(the tacrolimus group *vs.* betamethasone group), and ‡(the tacrolimus group *vs.* the ointment base group) denotes $P < 0.05$. **(b)** Representative pictures, illustrating different skin symptoms at the end of the observation period in each group.