



Anti-allergic effects of novel sulfated polysaccharide sacran on mouse model of 2,4-Dinitro-1-fluorobenzene-induced atopic dermatitis

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ABSTRACT

In this study, in order to investigate the potential of the novel polysaccharide sacran from *Aphanotece Sacrum* for development of atopic dermatitis (AD), we evaluated the potential of pretreatment with topical sacran to prevent the development of hapten (dinitrofluorobenzene: DNFB)-induced AD-like disease in mice. In the AD model mice, sacran markedly ameliorated AD symptoms such as scratching behavior and edema in ear. In addition, sacran significantly increased water content of the stratum corneum which regulates the skin barrier function. Furthermore, sacran significantly inhibited inflammatory cytokine and chemokine mRNA levels in the dermatitis skin as well as the IgE antibody level in serum. Sacran inhibited inflammatory cytokines mRNA production from Jurkat cells derived from human leukemia T cells after stimulation with phorbol 12-myristate 13-acetate/ionomycin. Meanwhile, sacran did not inhibit the proliferation of primary B cells stimulated with lipopolysaccharide. These results suggest that sacran has good anti-allergic effect on AD model mice, probably due to the amelioration of skin barrier function and suppression of immune responses.

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1. Introduction

Atopic dermatitis (AD) repeats the exacerbation and remission of symptoms such as pruritus and exanthema. AD is one of the most common chronic diseases in all over the world, especially in advanced country and characterized by exaggerated immune response and dry skin [1–5]. The cause of AD is considered environmental and genetic factors, however, the detail is unknown yet.

Commonly, the normal skin controls a barrier function by water evaporation from skin, and avoids antigens entering the body. However, the skin barrier function in AD patients is often broken. For example, the skin of AD patients increases transepidermal water loss (TEWL) and decreases the water content of stratum corneum due to the degradation of ceramide or cornified cell envelopes such as filaggrin, loricrin and involucrin, which are important to maintain the skin barrier [6,7]. Therefore, once antigens invade the fragile skin, the inflammatory reaction occurs. Therefore, the

both amelioration of skin barrier function and inhibition of immune responses are important for the treatment of AD.

There are a lot of therapeutic agents for AD such as steroids, immunosuppressive agents and anti-histamine agents. However, these agents infrequently cause regional side effects like atrophica cutis, capillary dilatation, moon face and osteoporosis. Therefore, novel therapeutic agents for AD symptoms without the adverse effects are necessary.

Sacran is a novel polysaccharide and extracted from the jelly-like extracellular matrix of a cyanobacterium, *Aphanotece sacrum*, a river alga mass-cultivated in Japan, and contains various sugar residues [8–10]. Previously, we demonstrated that sacran showed the potential anti-inflammatory effects for skin diseases models such as carrageenan-induced rat paw edema and 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced mouse ear edema through the suppression of mRNA expression of cyclooxygenase-2 (COX-2) as well as pro-inflammatory cytokines such as tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , and IL-6, without causing life-threatening adverse effects [11]. In addition, a concentration of 0.05% (w/v) sacran showed the most effective anti-inflammatory effects on skin disease model. Most recently, we conducted clinical study of 0.05% (w/v) sacran for the treatment of AD patients and revealed the amelioration of AD symptoms

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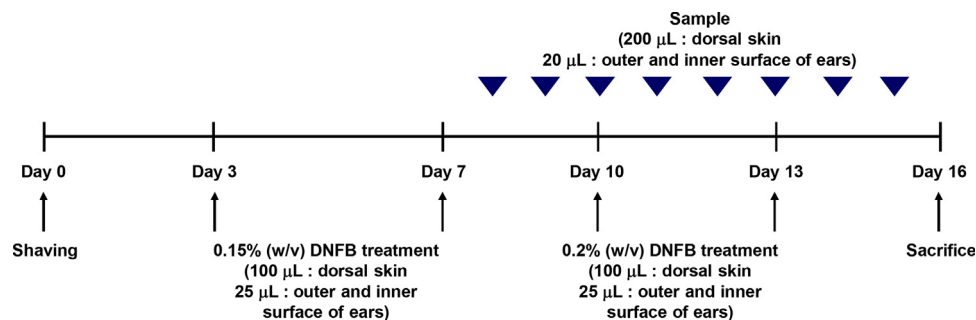


Fig. 1. Time Table for AD Model Mice Induced by DNFB.

AD was induced by repeated treatment of 100 µL of 0.15% (w/v) DNFB (acetone: olive oil = 3: 1) on dorsal skin of NC/Nga mice, and 25 µL of the same solution on both the outer and inner surfaces of the ears once daily on days 3 and 7, and then further apply with 0.2% (w/v) DNFB on days 10 and 13. The samples of 0.05% (w/v) sacran, 0.05% (w/v) CS and 0.5% (w/v) PD were applied to dorsal skin and surfaces of the ears.

[12]. However, the mechanism underlying sacran's efficacy on AD is still unknown. The objective of this study was to evaluate the potential of 0.05% (w/v) sacran as an anti-inflammatory agent for 2,4-Dinitro-1-fluorobenzene (DNFB)-induced AD model mice and to gain insight into the mechanism of its therapeutic effects.

2. Materials and methods

2.1. Materials

Sacran used in this study was newly extracted from cyanobacterium *Aphanothece sacrum* and was kindly gifted from Green Science Material (Kumamoto, Japan). Sacran is a heteropolysaccharide containing various sugar residues such as glucose, galactose, mannose, xylose, rhamnose, fucose, galacturonic acid and glucuronic acid, and traces of alanine, galactosamine and muramic acid; 11% of the monosaccharides contain a sulfate group and 22% of them contain a carboxyl group [9]. Sacran also has an extremely high molecular weight (approximately 20 MDa) and is surprisingly long (more than 8 µm) [9]. 2,4-Dinitro-1-fluorobenzene (DNFB) was purchased from Tokyo Chemical Industries (Tokyo, Japan). All other chemicals and solvents were of analytical reagent grade.

2.2. DNFB-induced allergic dermatitis in NC/Nga mice

NC/Nga mice (6 weeks of age, Japan SLC, Shizuoka, Japan) were sensitized by DNFB (Tokyo Chemical Industry Co. Ltd., Tokyo, Japan) recrystallized with ethanol. Briefly, 3 days and 7 days after shearing the dorsal skin of mice, 0.15% (w/v) DNFB solution in acetone-olive oil mixture (3:1) was epicutaneously applied on mice dorsal skin (100 µL) and ears (25 µL). Then, 10 days and 13 days later, the dorsal skin and ears of mice were challenged once with 100 µL and 25 µL of 0.2% (w/v) DNFB solution in acetone-olive oil mixture (3:1), respectively. NC/Nga mice were randomly divided into 5 experimental groups that received pretreatment a week after sensitization: normal control group, DNFB-treated group, 0.05% (w/v) sacran-treated group, 0.05% (w/v) chondroitin sulfate (CS)-treated group, and 0.5% (w/v) prednisolone (PD)-treated group. Blood was collected from the retroorbital plexus on day 14; the serum was obtained by centrifugation (1500 rpm, 15 min) and stored at −80 °C. Serum total IgE levels were measured using an immunoassay kit obtained from R&D Systems (Tokyo, Japan). All research adhered to the animal facility guidelines of Kumamoto University (A27-141).

2.3. Evaluation of scratching behavior episodes, the severity of dermatitis and ear thickness in mice

The number of videotaped scratching behavior episodes per unit time was counted with the use of a stopwatch on days 13. The contact between the hapten and the mouse ear skins induces itch that makes the animals scratch, and the more intense the itch, the more the number of scratching behavior episodes increases. We considered scratching behavior episodes that occurred within 30 min after exposure of mouse ears to the chemical allergen. The severity of skin lesions was evaluated macroscopically using the following scoring procedure for atopic/eczema dermatitis syndrome as previously described for dryness/scaling, erythema/hemorrhage, and excoriation/erosion: 0, no symptoms; 1, mild; 2, moderate; and 3, severe [13]. However, in this study, edema was indirectly evaluated through ear thickness measurement, and the mean severity scores of symptoms were calculated for each group of mice instead of the total. Skin symptoms were recorded on days 7–16. Edema was expressed as the difference between the baseline ear thickness and the ear thickness on days 7–16 using a dial thickness gauge (Teclock Corporation, Nagano, Japan).

2.4. Measurement of water content of the stratum corneum in dermatitis model mice

Water content of stratum corneum on dorsal skin of NC/Nga mice was measured with the use of Skin Moisture Sensor MY-808S (Scalar Corporation, Tokyo, Japan). Water content of stratum corneum was recorded on days 7–16.

2.5. Real-time PCR for the determination of mRNA levels of inflammatory biomarkers in dermatitis model mice

Total RNA was isolated from dorsal of dermatitis model mice, using TRIzol[®] Reagent according to the manufacturer's protocol. The RNA (0.5 µg) was subsequently reverse-transcribed using a ReverTra Ace[®] qPCR RT Master Mix (TOYOBO, Osaka, Japan). The mRNA expression levels of IL-4, IL-5, IL-31, IFN-γ, MCP-1, TNF-α, IL-1β and IL-6 were determined using a real time PCR assay. Real-time PCR was performed on a CFX96[™] Real-Time PCR (BIO RAD, Tokyo, Japan) using 1 µL of cDNA for each sample. THUNDERBIRD[™] SYBR[®] qPCR Mix (TOYOBO, Osaka, Japan) was used to detect products, and 10 µM concentrations of the following primers were used: mouse GAPDH forward: 5'-GGTGAAGGTCCGTGTGAACGGATT-3', mouse GAPDH reverse: 5'-AATGCCAAAGTTGTGATGGATGACC-3', mouse IL-4 forward: 5'-TCGGCATTGTTGACACGAGGTC-3', mouse IL-4 reverse: 5'-GAAAAGCCCGAAAGAGTATC-3', mouse IL-5 forward: 5'-ATGGAGATTCCCATGAGCAC-3', mouse IL-5 reverse: 5'-GTCTCTCCTCGCCACACTTC-3', mouse IL-31 for-

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