



Anti-inflammatory effects of sacran, a novel polysaccharide from *Aphanothece sacrum*, on 2,4,6-trinitrochlorobenzene–induced allergic dermatitis in vivo

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ABSTRACT

Background: Sacran is a newly discovered sulfated polysaccharide extracted from an algae, *Aphanothece sacrum*, grown in a river of the Kyushu region in Japan.

Objective: To evaluate sacran's inhibitory effect in 2,4,6-trinitrochlorobenzene (TNCB)–induced allergic dermatitis in NC/Nga mice.

Methods: Sacran was extracted by acid and alkaline treatment of *A sacrum* cyanobacterial biomaterials. To sensitize mice, 150 μ L of 5% TNCB was applied epicutaneously on the abdomen of each mouse on day 1 and challenged with 15 μ L of 1% TNCB applied on the ear skin of mice on day 8 and then every other day to induce skin lesions. Serum levels of inflammatory markers were measured and histopathologic examination of ear skin specimens performed. On the other hand, sacran's transepidermal water loss was evaluated in 11 volunteer women with dry skin.

Results: Epicutaneous application of sacran in mice has significantly inhibited the development of allergic dermatitis skin lesions and reduced the number of scratching behavior episodes ($P < .01$). In addition, sacran efficiently inhibited IgE ($P < .001$), tumor necrosis factor α ($P = .02$), interleukin 4, interleukin 5, and interferon γ ($P < .01$; vs buffer in the TNCB group) production and eosinophilic infiltration in the chemical allergen–exposed ear skin. In addition, sacran-treated body regions of human volunteers with dry skin significantly reduced transepidermal water loss levels compared with exogenous hyaluronic acid ($P < .01$), which is known to improve skin moisture and exert skin barrier repair activity.

Conclusions: This study suggests that sacran exerts anti-inflammatory effects by improving skin barrier function and reducing T_H2 cytokine production.

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Introduction

Environmental and occupational allergic dermatitis have become serious health issues in most developed countries, with increasing incidence. In the United States, contact dermatitis is one of the most prevalent occupationally related illnesses and incurs costs of approximately \$1 billion annually.¹ Severe itching is the most important problem, causing impairment of patient quality of life.²

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New natural products that can control the symptoms related to allergic dermatitis without the adverse effects of steroids would be good alternatives for the treatment.^{2–6}

Sacran is extracted from *Aphanothece sacrum*^{7,8} and contains various sugar residues, including Glc, Gal, Man, Xyl, Rha, Fuc, GalA, GlcA, and traces of Ara, GalN, and Mur; 11% of the monosaccharides contain a sulfate group and 22% contain a carboxyl group. *A sacrum* is a mass cultivated cyanobacterial species that has been used as a functional food to improve allergic tendency and gastroenteritis by inhabitants of the Kyushu region in Japan.

We previously investigated the physical properties of sacran, such as its strong ability to adsorb metal ions and its efficient liquid crystallization.^{8,10} However, no reports exist in the medical literature on the beneficial health effects of *A sacrum*, particularly its polysaccharides. The objectives of this study were to improve

sacran's extraction method to obtain highly soluble biomedical materials that could be used in vivo and to evaluate its potential anti-inflammatory effects in 2,4,6-trinitrochlorobenzene (TNCB)-induced allergic dermatitis in mice.

Methods

Extraction, preparation, and biochemical analyses

We modified the methods described in the supporting information section of the previous report⁹ for extraction and purification of sacran from *A sacrum* biomaterials. The *A sacrum* biomaterials (wet weight, 1 kg), which were picked up from a river farm (scale, 100 tons per year) on the Kyushu island of Japan, were freeze-thawed, washed in distilled water to remove aqueous pigment (phycobilin), immersed and stirred in a water bath filled with a large amount of dilute hydrochloric acid (pH 3) at 60°C for 24 hours, washed with 50% to 70% (vol/vol) isopropanol overnight, and then collected by filtration and air dried with a drying machine at 60°C. The polysaccharide was extracted using 0.1M sodium hydroxide solution at 70°C with agitation for 3 hours by mechanical motor stirrer with a rabbler.

The solution containing polysaccharides was adjusted to a pH of 7 to 8 by 0.1M hydrochloride and filtered by sterile cotton gauge. The filtrate was slowly poured into absolute isopropanol to precipitate white fibrous materials, which were then redissolved in hot water and reprecipitated 3 times. The polysaccharide fibers were dried (yield, 0.65–0.75 wt% to wet biomaterials and 60–70 wt% to dried ones). The aqueous solution showed no specific absorption in the wavelength range of 220 to 600 nm in UV-visible spectroscopy, which confirmed that there was no contamination by proteins, nucleic acid, chromospheres, and/or other chemicals with UV-visible absorption. The sacran solution used in this experiment was prepared by dissolving the dried polysaccharides in hot water (80°C) at a concentration of 2% (wt/vol).

Fourier-transformed ion cyclotron resonance mass spectroscopy (FT-ICR-MS), gas chromatography–mass spectroscopy (GC-MS), and viscosity measurement were performed by the previously reported methods.⁹

NC/Nga mice, treatments, and disease induction

Twenty-four inbred male NC/Nga mice (6 weeks of age) were purchased from Japan SLC (Shizuoka, Japan). Animals were housed under conventional conditions at the animal facility of Kochi Medical School, Kochi, Japan. All research adhered to the animal facility guidelines of Kochi Medical School (C000144).

To induce allergic dermatitis skin symptoms, TNCB (Nacalai Tesque Inc., Kyoto, Japan) was used after recrystallization with ethanol. To sensitize mice, 150 μ L of 5% TNCB was applied epicutaneously on mice abdomen, at 7 weeks of age, as previously described.^{12,13} A week later (day 8), the dorsal surfaces of mice ears were challenged once with 15 μ L of 1% TNCB solution, obtained after dissolution of 5% TNCB in acetone-olive oil mixture (4:1). Mice were again challenged with 1% TNCB every other day until day 20 (eFig 1).

Vials (1 g) of water-soluble hydrocortisone were obtained from Nacalai Tesque Inc (Kyoto, Japan) and diluted in deionized double distilled water, after recrystallization with ethanol, to a final concentration of 10 mg/mL for topical use in animal experiment as previously described.¹¹ NC/Nga mice were randomly divided into 4 experimental groups that received pretreatment a week after sensitization: sacran-pretreated group, hydrocortisone-pretreated group, buffer-pretreated group, and normal control group. Mice that received pretreatment were always anesthetized with the use of diethyl ether by inhalation before pretreatment and challenge to TNCB.

On day 21, ear biopsies were performed and skin specimens fixed in 10% formalin, embedded in paraffin, sectioned at 10 μ m, and stained with hematoxylin-eosin. To examine the skin specimens, light microscope BX60 (Nikon, Japan) was used at a $\times 100$ magnification. Blood was collected from the retroorbital plexus on day 21; the serum was obtained by centrifugation (3,000g, 30 minutes, 4°C) and stored at -80°C .

Serum levels of total IgE, tumor necrosis factor α (TNF- α), interferon γ (IFN- γ), interleukin (IL) 4, IL-5, eotaxin, and monocyte chemoattractant protein 1 (MCP-1) were measured using immunoassay kits obtained from R&D Systems (Tokyo, Japan) and following the manufacturer's instructions.

Evaluation of scratching behavior episodes and the severity of dermatitis in mice

The number of videotaped scratching behavior episodes per unit time was counted with the use of a stopwatch on days 8, 14, and 20. The contact between the hapten and the mouse ear skin 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 10 minutes after exposure of mouse ear 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 8, 14, and 20.

Statistical analysis

All data were expressed as mean $\pm$ SD. Statistical significance of differences in the severity of dermatitis, serum levels of biomarkers, and TEWL levels was assessed by 1-way analysis of variance followed by the unpaired *t* test. The difference in scratching behavior episodes was analyzed by the *t* test. *P* < .05 was considered statistically significant. All analyses were performed using Stata software, version 10 (Stata Corp, College Station, Texas).

Results

Extraction of sacran

When extracted by the alkaline dissolution of acid-washed *A sacrum*'s biomaterials, sacran showed low contents of multivalent iron, aluminum, and magnesium ions, which bind strongly to sacran chains. The decrease in metal content raised the solubility of sacran in water to prepare a 10% (wt/vol) solution, whereas sacran extracted by the method previously reported^{8–10} (without acid treatment) dissolved in water up to a concentration of only 1.5 wt%. In addition, the molecular weights of sacran extracted by the present method were reduced compared with the previously reported method. The improved solubility may be attributed to an attenuation of metal-mediated aggregation. The improved solubility may be attributed to an attenuation of metal-mediated aggregation. To determine whether acid treatment produced a molecular weight decrease after the acid treatment, gel permeation chromatography measurements of sacran were performed to detect a monomodal gel permeation chromatography peak of molecular weight = 1.24×10^7 and number average molecular weight = 5.30×10^6 . The molecular weights of sacran extracted by the present method were reduced compared with the previous one.

Table 1
Mass/charge (m/z) values of main peaks and corresponding sugars^a

Entry	m/z	Calculated m/z	Sugar constituents	Molecular weight	Measurement mode
1	355.0869	356.095 [M-H]	Uronic acid + Glc or Gal	365.095	Negative
2	369.0667	370.075 [M-H]	Uronic acid + uronic acid	370.075	Negative
3	545.162	546.144 [M-H]	Methylated (uronic acid + uronic acid + Gal or Glc)	546.143	Negative
4	693.2089	694.217	Methylated (uronic acid + Gal + Glc + hexose)	694.217	Negative
5	301.0239	301.023 [M-H]	Sulfated dimethyl uronic acid	302.031	Negative
6	358.0819	359.09 [M-H]	Sulfated dimethyl muramic acid	359.089	Negative
7	435.1127	435.112 [M+Na] ⁺	Dimethylated (uronic acid + uronic acid)	412.122	Positive
8	463.0773	464.076 [M-H]	Sulfated dimethylated (uronic acid + Gal + Glc)	464.084	Negative
9	630.21	630.224 [M-H]	Methylated (Glc + Gal + N-acetyl muramic acid)	631.232	Negative

^aAfter performing the gas chromatography–mass spectroscopy study of hydrolysate sacran, which allowed knowing the main constituent sugars (Glc, Gal, Man), Fourier-transformed ion cyclotron resonance mass spectroscopy analysis was performed to determine the composition of oligosaccharides using the m/z values of the main peaks.

FT-ICR-MS analyses of sacran structure

We previously reported that sacran contained more than 9 monosaccharides as constituent sugars using GC-MS analyses, namely, Glc, Gal, Man, Xyl, Rha, Fuc, GalA, and GlcA, with composition ratios of 25.9, 11.0, 10.0, 16.2, 10.2, 6.9, 4.0, and 4.2.⁹ The FT-ICR-MS of methanolized sacran demonstrated the presence of sulfated muramic acid, a unique sugar found only in sacran thus far. FT-ICR-MS also revealed the sequence of muramic acid connecting to hexose, Glc, and Gal. These results demonstrated that the muramic acid is not a constituent of cell wall peptide glycan, where the muramic acid never connects to hexose, but rather a constituent of the capsular polysaccharides of *A. sacrum*.

To determine its detailed primary structure, sacran was partially hydrolyzed in acid to give mixtures of oligomers, and partial structures were analyzed by a millimass estimation of the obtained oligomer mixtures. Initially, the dry sample of sacran (0.5 g) was hydrolyzed with 4M trifluoroacetic acid (50 mL) for 21 hours at 121°C, and during hydrolysis treatment, we repeatedly checked the decomposition using thin-layer chromatography analyses to confirm the presence of monosaccharides, disaccharides, and trisaccharides. GC-MS studies of the hydrolysate indicated that the main constituent sugars of the sacran fraction were Glc and Gal, and the Man content was negligibly low. The molecular weights of the hydrolysate were low (below 700) enough to analyze by FT-ICR-MS.

Next, the reducing terminals of the obtained products were methylated with methanol (50 mL)/acetyl chloride (3.6 mL) to measure their mass by FT-ICR-MS. Compositions of oligosaccharides were analyzed based on mass/charge values of main peaks. For example, in entry 5 of Table 1, the measured value of 301.0239 corresponds with the calculated one for a [M-H][−] (301.023) from sulfated dimethyl uronic acid on the level of millimass units, and other corresponding compositions were not found even if various combination of sugars, substituents, and ionizations were used in calculations. Thus, the peak was assigned to sulfated dimethyl uronic acid. Eight other sugar constituents found by the same method are indicated in Table 1.

The common sequence entries of 1, 2, 3, 7, and 8 contain an uronate diad, whereas the common sequence entries 6 and 9 contain muramic acid plus hexoses, such as Glc and Gal. It is presumed that these sequences are built into the polysaccharides because each peak intensity was high. In addition, we found a sulfated hexose signal [M-H][−] = 259.012. From Table 1, we extracted 2 trisaccharide sequences and determined a partial structure of sacran chain, where sulfate groups were delocalized and attached at muramic acid, uronic acid, and hexose (Fig 1).

Viscosity of the sacran solution

The sacran solution showed a shear-thinning effect; the viscosity of sacran solutions decreased with an increase in shear rate. We evaluated this shear thinning over a 10-fold increase in shear rate,

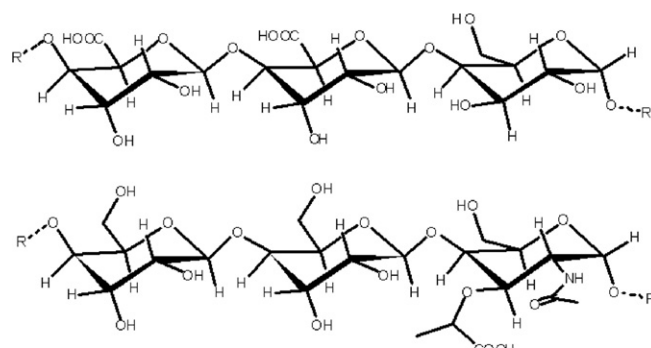
and sacran solutions with concentrations of 0.06, 0.13, 0.25, 0.5, 1.0, 1.5, 2.0, 5.0, and 10.0 wt% showed 3.3-, 3.8-, 4.1-, 4.5-, 5.5-, 6.4-, 6.7-, 8.0-, and 11.1-fold shear thinning, respectively. This viscosity may be attributed to (1) the interchain sliding of sacran chains, which have a repulsive interchain interaction due to the sulfate and carboxylate negative charges, and (2) an efficient liquid chromatography orientation along the shear flow. The high shear-thinning effects (atomic force microscopy images) of sacran concentrations higher than 0.5 wt% have an advantage in generating an efficient coating on the injury and provide sufficient moisture.

Effect of sacran on the development of allergic dermatitis in mice

As early as week 1 of allergic dermatitis induction, all TNCB mice developed skin lesions, predominantly moderate to severe scaling/dryness and erythema/hemorrhage. Conversely, the development of those lesions was inhibited by the topical application of sacran and hydrocortisone in the corresponding mice groups (Fig 2).

At the end of the experiment, after a total of 6 challenges to 1% TNCB, significantly lower severity scores were observed in both the sacran and hydrocortisone groups for scaling/dryness, erythema/hemorrhage ($P < .01$), and excoriation/erosion ($P < .001$), compared with the TNCB group.

A significantly lower number of scratching behavior episodes was noted in the sacran ($P < .001$) and hydrocortisone ($P = .02$) pretreated mice compared with the TNCB group (Fig 3). In addition, 2% sacran showed a relatively better inhibitory effect compared with hydrocortisone ($P = .02$). Furthermore, sacran-pretreated mice showed no significant difference in terms of scratching behavior count compared with buffer-treated normal control mice ($P > .05$).



Sacran polysaccharide

Fig. 1. Partial chemical structure of sacran determined by Fourier-transformed ion cyclotron resonance mass spectroscopy. A partial structure of sacran chains was found, which was obtained after hydrolyzation in acid to determine the oligomers that were analyzed by a millimass estimation of the oligomer mixture.

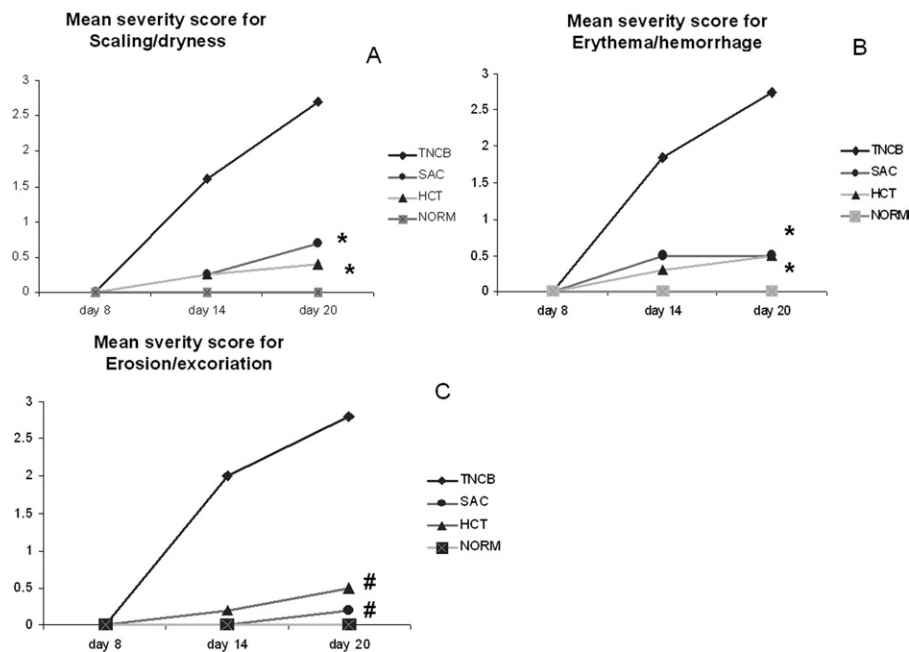


Fig. 2. Distribution of mean severity scores of skin symptoms in mice. A, Scaling/dryness; B, erythema/hemorrhage; C, erosion/excoriation. Sacran (SAC) and hydrocortisone (HCT) significantly inhibited the development of scaling/dryness ($P < .001$), erythema/hemorrhage ($P < .001$), and erosion/excoriation ($P < .001$) after repeated challenges to the allergen (2,4,6-trinitrochlorobenzene [TNCB]) compared with the TNCB group. * $P < .001$ by 1-way analysis of variance (ANOVA) followed by the unpaired t test (compared with the TNCB group). # $P < .001$ by 1-way ANOVA followed by the unpaired t test (compared with the TNCB group). NORM indicates normal control mice group.

Serum levels of IgE, T_H1/T_H2 cytokines, MCP-1, and eotaxin in mice

Significantly lower serum levels of total IgE were noted in 2% sacran and 10 mg/mL of hydrocortisone-pretreated mice ($P < .001$) compared with the TNCB group, and no significant difference was found in serum total IgE titers between the sacran and hydrocortisone groups ($P = .55$) (Fig 4). Also, the levels of serum total IgE in the sacran-pretreated mice correlated with the severity scores of allergic dermatitis skin symptoms.

With respect to $TNF-\alpha$ ($P = .02$), $IFN-\gamma$ ($P < .01$), and IL-5 ($P < .01$), the sacran group had significantly lower serum levels compared with the TNCB group, whereas the hydrocortisone-pretreated group showed significantly lower levels for IL-5 and $IFN-\gamma$ ($P < .01$) but not $TNF-\alpha$ ($P = .12$). However, the serum levels of IL-4 were too low and undetectable in sacran, hydrocortisone, and the normal control groups but were increased in the TNCB mice (Fig

5A). This finding suggests that sacran and hydrocortisone pretreatments suppressed IL-4 production. Furthermore, sacran mice had significantly lower serum MCP-1 and eotaxin ($P < .001$) levels than the TNCB group (Fig 5B).

Macroscopic and histologic features of mice ear skin specimens

At the end of the experiment, macroscopic analysis of ear skin revealed the presence of hyperkeratosis and moderate to severe skin lesions in all TNCB mice, whereas only mild or nonmacroscopically visible lesions were observed in the sacran and hydrocortisone-pretreated mice.

Histologic analysis of the hematoxylin-eosin-stained sections of ear skin showed skin barrier disruption and dense infiltration of inflammatory cells (mostly eosinophils and lymphocytes), with

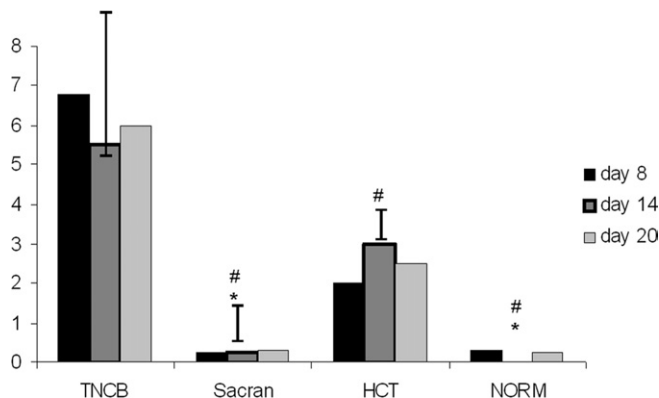


Fig. 3. Distribution of the mean number of scratching behavior episodes in mice. A significant inhibition of itch or scratching behavior was noted in the 2% sacran-pretreated mice (sacran) ($P < .001$) and 10-mg/mL hydrocortisone-pretreated mice (HCT) ($P = .02$) compared with the 2,4,6-trinitrochlorobenzene (TNCB) mice (negative control). Sacran's itch inhibitory effect was greater compared with HCT ($P = .02$). NORM indicates normal control mice group. * $P < .05$ (sacran vs HCT; HCT vs TNCB; NORM vs HCT). # $P < .001$ (sacran vs TNCB; NORM vs TNCB).

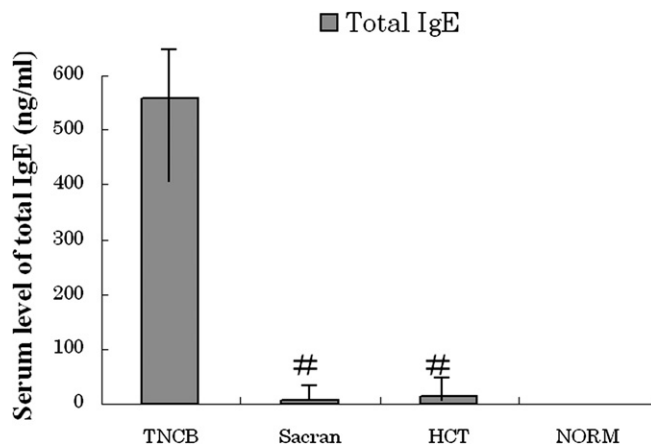


Fig. 4. Serum levels of total IgE in mice. The serum levels of total IgE were significantly lower in the sacran and hydrocortisone (HCT) ($P < .001$) pretreated groups compared with buffer in the negative control (2,4,6-trinitrochlorobenzene [TNCB]) group. No statistically significant difference was noted between the sacran and HCT groups ($P = .55$). NORM indicates normal control mice group. # $P < .001$ by the t test (sacran vs TNCB; NORM vs TNCB).

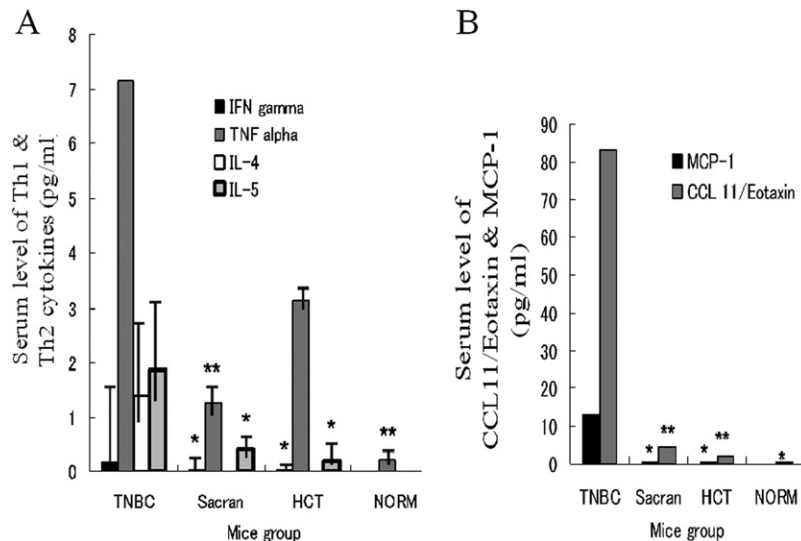


Fig. 5. Serum levels of T_H1 (tumor necrosis factor α [TNF- α] and interferon γ [IFN- γ]), T_H2 (interleukin [IL] 4 and IL-5), cytokines (A), monocyte chemoattractant protein 1 (MCP-1), and eotaxin (B) in mice. Significantly lower serum levels of TNF- α ($P = .02$), IL-4, IL-5, and IFN- γ ($P < .01$) were found in sacran-pretreated mice group compared with the 2,4,6-trinitrochlorobenzene (TNBC) group. Lower levels of IL-5 and IFN- γ ($P < .01$) were also found in the hydrocortisone-pretreated group. Serum levels of IL-4 were undetectable in the sacran, hydrocortisone (HCT), and normal (NORM) control mice groups. In addition, the serum levels of MCP-1 ($P < .01$) and eotaxin ($P < .001$) were markedly lower in the sacran and HCT groups (vs the TNBC group). * $P < .05$.

hemorrhagic zones in TNBC (negative control) mice. However, these processes were inhibited in sacran-treated mice. A reepithelization of ear skin was also noted in the sacran-treated mice (Fig 6).

Discussion

This study highlights some of beneficial effects of sacran polysaccharide, suggesting that it has particular health benefits compared with other sulfated polysaccharides. The extraction of sacran by alkaline dissolution of acid-washed bacteria yielded a product with improved solubility, with a reduction in molecular weight. The improved solubility appears to be due to an associated reduction in metal ions. The alkaline-extracted sacran was shown to be beneficial in enhancing the skin barrier function and preventing the development of TNBC-induced allergic dermatitis in mice.

The dry itchy skin and the elevated serum IgE level are associated with the disease severity and the increase in scratching behavior episodes.¹¹ The latter causes mechanical skin injury, leading to cytokines and chemokines release.^{12–14} Sacran displayed a good itch-inhibiting effect that led to reduced scratching behavior episodes and also significantly inhibited the production of IgE, T_H2

(IL-4 and IL-5), and T_H1 cytokines (TNF- α and IFN- γ) in mice. Furthermore, sacran inhibited inflammatory cell chemotaxis in the hapten-exposed ear skin. The *in vivo* topical application of sacran has also markedly reduced transepidermal water loss (eMethods) in individuals with dry skin; its skin barrier protective effect was more efficient than that of hyaluronan, a well-known polysaccharide that is reported to improve skin moisture and exerts skin barrier repair activity.¹⁵ This finding suggests that sacran has a good effect on skin barrier protection and repair.

The main reason for the anti-inflammatory effect displayed by sacran is an enhanced stability of the skin barrier induced by sacran polysaccharide, inhibiting the release of inflammatory cytokines and chemokines that are believed to be responsible of the inflammatory process and the development of skin allergy, a condition that is generally characterized by the increased concentration of serum IgE and eosinophilia.^{16,17} In addition, results from this allergic dermatitis prophylactic study showed that topical application of 2% sacran significantly inhibited the production of IgE.

The increase in the serum IgE level in patients with atopy or eczema, which is reported to occur in 80% of these patients, is

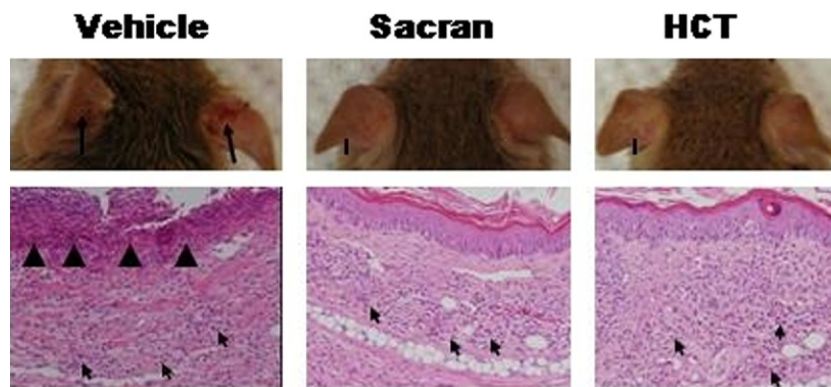


Fig. 6. Macroscopic and histologic analysis (hematoxylin-eosin stain) of ear skin specimens of mice. Black triangles indicate the complete disruption of the skin barrier in the 2,4,6-trinitrochlorobenzene (TNBC) mouse; thick bars show ear skin lesions (severe excoriation et hemorrhage in the TNBC mouse, mild scaling in the sacran and hydrocortisone [HCT] mice); arrows show presence of cell (eosinophils, lymphocytes) infiltrates. Hyperkeratosis and hypertrophy of ear skin severe skin lesions were found in the TNBC (negative control) mouse. Hematoxylin-eosin-stained sections of ear skin reveal a disruption of skin barrier and a dense infiltration of inflammatory cells (mainly eosinophils and lymphocytes), with hemorrhagic zones in the TNBC or negative control mice, whereas sacran and HCT inhibited these processes.

closely related to clinical symptoms and the production of T_H2 cytokines.^{18,19} This relationship was observed in the sacran-pretreated group in our experiment, where lower levels of total serum IgE and inhibition of the development of clinical symptoms were found.

Previous studies suggest that skin lesions accompanied by eosinophilia and systemic IgE elevation are associated with T_H2 -type cytokine expression.²⁰ On the other hand, although the overproduction of T_H2 cytokines is known to be a characteristic of skin allergic disease (including atopic dermatitis), the production of T_H1 cytokines may also increase in animal model of the disease, which is thought to be in accordance with the description of a chronic phase of the allergic skin inflammation.^{20–22} Our experiment showed that sacran-pretreatment inhibited the production of T_H2 cytokines (IL-4, IL-5), T_H1 cytokines (TNF- α , IFN- γ), and inflammatory chemokines, such as MCP-1 and eotaxin, thus inhibiting the induction of inflammatory process.

The present study improved sacran's extraction method, increasing its solubility by reducing its metal content; it also demonstrated sacran's skin barrier repair activity and showed the anti-inflammatory effects of sacran. Several beneficial effects of plant polysaccharides, such as fucoidin and fucoidan, have been demonstrated on human health,^{23–29} especially in regard to their antioxidant, antitumor activity and preventive anti-inflammatory activities. Our recent works reveal that topical sacran not only has an efficient skin repair skin barrier activity but also ameliorates skin elasticity and skin hydration in a coreometric study (data not shown in this report) and efficiently improves atopic dermatitis-like skin lesions in diseased mice in a curative protocol.³⁰ Those properties displayed by sacran are not common to most sulfated polysaccharides.

In conclusion, results from this study suggest that sacran, a unique cyanobacteria-derived glycosaminoglycanoid, exerted an antiallergic activity mainly by improving the skin barrier and downregulating T_H2 cytokine production, leading to inhibition of IgE and eosinophilic infiltration in mice. Thus, sacran might potentially be beneficial in the treatment of allergic dermatitis. Further research is ongoing to determine the inflammatory pathways involved and evaluate sacran's curative effects on allergic dermatitis in humans compared with other therapeutic agents.

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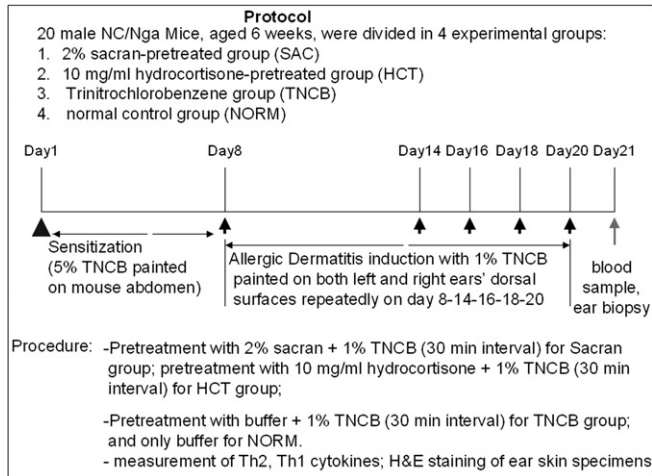
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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.anai.2011.10.013.

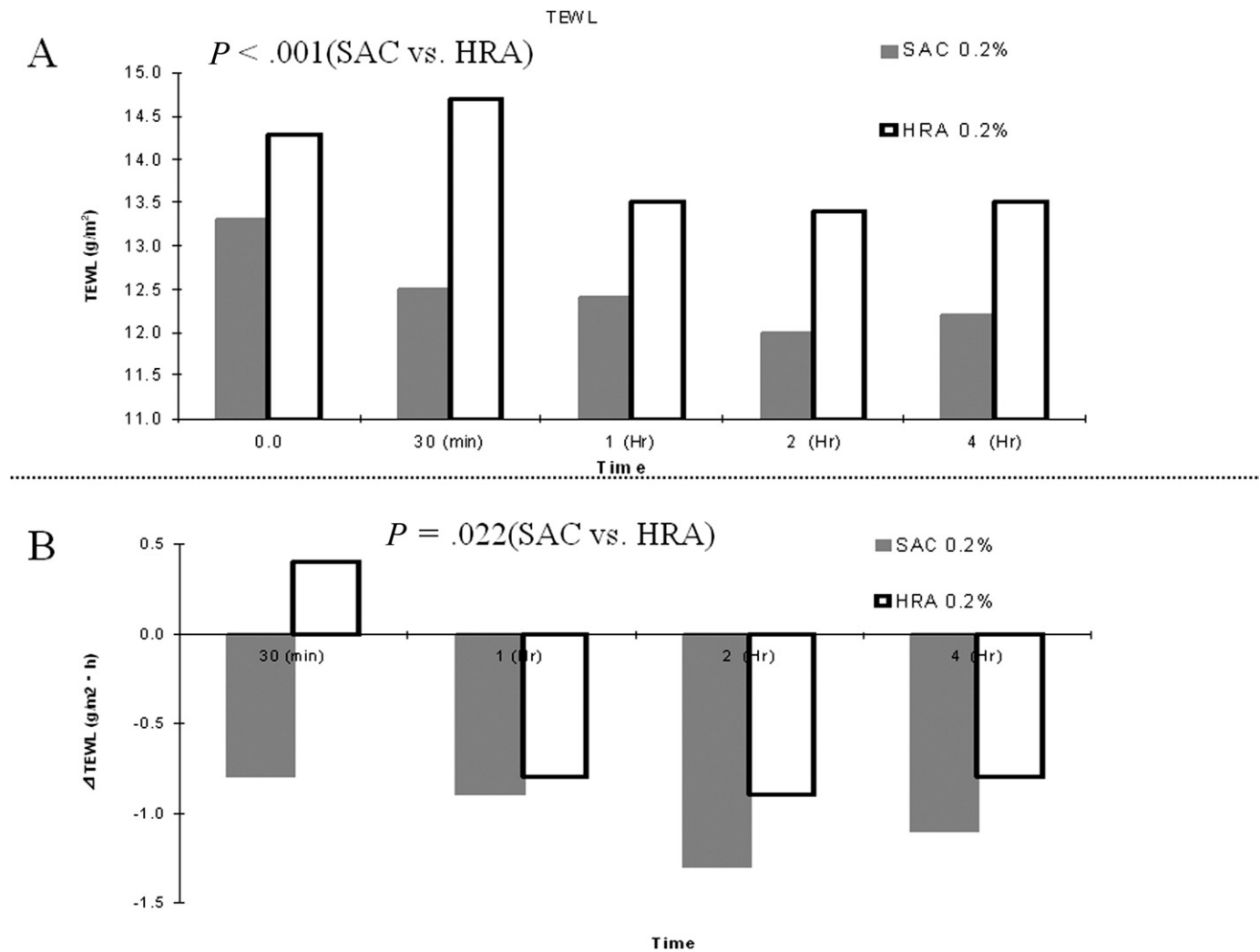


eFigure 1. Animal experiment protocol. H&E indicates hematoxylin-eosin.

eMethods. Transepidermal Water Loss (TEWL) in human volunteers with dry skin

Dry skin is a condition characterized by impaired skin barrier function that can be found in a number of diseases, including atopic dermatitis and senile eczemas (Isoda et al, 2011). Eleven female Asian volunteers with dry skin, aged 45 to 60 years, participated in the investigation, and TEWL was evaluated at 30 minutes, 1 hour, 2 hours, and 4 hours using the VapoMeter Wireless SWL4 (Delfin Technology, Kuopio, Finland) (Fluhr et al, 2005; Pinnagoda et al, 1990) after treatment with sacran or hyaluronic acid. They were asked to refrain from applying any other topical formulations to the treated body areas (forearms) during the experiment. The treated dry skin-affected body region on each participant was divided into 2 areas ($8 \times 6 \text{ cm}^2$) and randomized for the treatment: area 1 for 0.2% sacran (SAC) and area 2 for 0.2% of exogenous hyaluronic acid (HRA). Written informed consent was obtained from each participant.

TEWL measurement is useful for identifying skin damage caused by certain chemicals or pathological conditions, such as eczema or allergic dermatitis, with TEWL rates increasing proportionally to the level of skin damage. The topical application of sacran on dry skin patients significantly reduced TEWL levels compared with hyaluronic acid ($P < .001$). Sacran reduced TEWL and ΔTEWL ($P = .02$) throughout the investigation, and its skin barrier repair activity was more efficient than that of hyaluronic acid (eFig 2).



eFigure 2. Transepidermal water loss (TEWL) levels in humans with dry skin. Significantly lower levels of (A) TEWL ($P < .001$) and (B) Δ TEWL ($P = .02$) were found in sacran (SAC)–pretreated body areas of individuals with dry skin compared with hyaluronic acid (HRA), suggesting that sacran repaired the skin barrier more efficiently than HRA did. P values (TEWL; sacran vs HRA) were determined by 1-way analysis of variance followed by an unpaired t test.