Regular Article

Defensive Effects of a Unique Polysaccharide, Sacran, to Protect Keratinocytes against Extracellular Stimuli and Its Possible Mechanism of Action

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Sacran, a polysaccharide isolated from the alga *Aphanothera sacrum* (Suizenji-nori), has unique physical and physiological characteristics. In a previous study, we reported that sacran improves skin conditions in individuals who suffer from atopic dermatitis (AD), focusing on its trapping function against extrinsic stimuli compared with hyaluronic acid (HA). First, we examined the penetration of sacran through stratum corneum (SC) with an impaired barrier function using immature reconstructed human epidermal equivalents. Sacran penetrates the SC to living cell layers of the epidermis, which suggested that sacran would attenuate adverse influences in keratinocytes caused by extracellular factors such as irritants or proinflammatory cytokines such as interleukin 1 α (IL-1 α). Sacran markedly reduced the cell damage induced by a nonionic detergent, sodium lauryl sulfate (SLS). Moreover, sacran restored the elevation of intracellular reactive oxygen species (ROS) levels stimulated by SLS and by IL-1 α . These effects of sacran were superior to those of HA. In order to investigate the restoration effects of sacran, the influence of sacran on the physical properties of lipid bilayers was evaluated by measuring the order parameter using the ESR spin-labeling method. Because sacran failed to cause changes in the order parameters of liposomes and HaCaT keratinocytes, these results indicate that sacran does not interact with lipid bilayers although it restored changes in the order parameter caused by SLS. The sum of these results demonstrates that sacran reduces the influence of extracellular stimuli by its trapping effects. We conclude that the improving action of sacran is based on its trapping effect.

Key words sacran; polysaccharide; extracellular stimuli; sodium lauryl sulfate (SLS); trapping effect

Rough skin is characterized by low hydration and high levels of trans-epidermal water loss (TEWL) as physiological parameters and scaling is often observed. In general, rough skin is likely to occur in subjects who are exposed chronically to low temperature and/or low humidity, as occurs in the winter season, or to chemicals such as detergents. On the other hand, although atopic dermatitis (AD) is a substantially allergic disease, the characteristics of physiological skin parameters in patients with AD include a lower epidermal barrier function. Indeed, dysfunction of the filaggrin gene has been shown to be involved in the disruption of the barrier function and in the genetic pathogenesis of AD.^{1,2} Even healthy skin is affected by the influence of air pollutants such as PM 2.5 (particulate matter 2.5). Although environmental changes in our life have not been identified as a causative factor, people who suffer from hyper-susceptibility to sensory irritation have an increased risk of sensitive skin.³ Sensitive skin has also been reported to have the characteristic of a lower epidermal barrier function.⁴ The sum of these facts suggests that the improvement of epidermal barrier function is an important task considering that rough skin has conditions similar to the mild symptoms of AD skin.

Sacran, a polysaccharide isolated from the alga “*Aphanothera sacrum*” (Suizenji-nori), is composed of 11 kinds of saccharides, including sulfate and carboxylic acid groups.^{5–7} Because of its unique structure, sacran has a high-water retention compared with hyaluronic acid (HA).⁶ It has been reported that sacran also has a unique physical property in that it can form a gel-like film in combination with polyols and such a

film can decrease the water evaporation rate and the penetration of calcein like an artificial barrier.⁸ Furthermore, a serum containing sacran can improve skin conditions both in healthy subjects and in patients with AD.^{9,10} Considering these facts, it is expected that sacran works not only as an artificial barrier on the skin surface but also penetrates to living epidermal cells to improve skin conditions. Thus, we hypothesized that sacran can act as another artificial barrier in the epidermis to maintain or improve skin conditions.

Rough skin can be experimentally reproduced by the topical application of sodium lauryl sulfate (SLS), which is a representative anionic surfactant.¹¹ Repeated application of SLS on the skin induces lower skin hydration and a higher level of TEWL, in addition to the appearance of scaling.^{12,13} As alterations of parameters derived from corneocytes, a high ratio of interleukin (IL)-1 α receptor antagonist and IL-1 α and a high level of IL-8 have been observed.¹⁴ Furthermore, dry skin in the winter season contains carbonylated proteins at a high frequency. A recent study demonstrated that SLS easily penetrates into the epidermal living cell layers¹⁵ and increases intracellular reactive oxygen species (ROS) levels and the secretion of IL-1 α . IL-1 α also stimulates ROS generation in HaCaT keratinocytes.¹⁶

Thus, this study was conducted to assess the effects of sacran to improve skin conditions characterized by impaired epidermal barrier function, such as occurs in patients with AD, focusing on its trapping function against extrinsic stimuli into the epidermis and to determine its mechanism of action. In this study, we used SLS as a major extrinsic stimulant and

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examined the effects of sacran on the influence of SLS and IL-1 α , which is secreted following treatment with SLS, on HaCaT keratinocytes.

MATERIALS AND METHODS

Reagents Sacran was extracted from the alga "*Aphanothera sacrum*" (Suizenji-nori) and was purified at DAITO KASEI KOGYO (Osaka, Japan). HA, SLS, α -tocopherol, lecithin from soybeans and cholesterol were purchased from Nacalai Tesque (Kyoto, Japan). Dulbecco's modified Eagle's medium (DMEM) and Hanks' Balanced Salt solution with Ca²⁺ and Mg²⁺ (HBSS) were obtained from Nissui Pharmacy (Tokyo, Japan). Fetal bovine serum (FBS) was obtained from Invitrogen (Carlsbad, CA, U.S.A.). The BCA Protein Assay Reagent kit was purchased from Pierce Chemical Co. (Rockford, IL, U.S.A.). The IL-1 α , IL-1 α enzyme-linked immunosorbent assay (ELISA) Quantikine kits and streptavidin-horseradish peroxidase (HRP) were purchased from R&D Systems (Minneapolis, MN, U.S.A.). 5-Doxyl stearic acid (5-DSA) and 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride were purchased from Sigma (St. Louis, MO, U.S.A.). LabCyte EPI-MODEL, reconstructed human epidermal equivalents (RHEEs) at 6d and 12d, and their culture media were obtained from Japan Tissue Engineering (Aichi, Japan). 6-[6-(Biotinylamino)hexanoylamino]-hexanoylhydrazine (biotin-AC5-hydrazide) and Hoechst33342 solution were obtained from Dojindo Laboratories (Kumamoto, Japan). DyLight650-labeled streptavidin was obtained from Thermo Fisher Scientific (Waltham, MA, U.S.A.). 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)diammonium salt (ABTS) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Biotin Conjugation to Polysaccharides Polysaccharides were reacted with 2mg/mL biotin-(AC5)2-hydrazide in the presence of 20mg/mL 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride and pyridine for 5h. Biotin-conjugated polysaccharides were purified by dialysis against de-ionized water.

Dynamics of the Penetration of Polysaccharides into RHEEs RHEEs were treated topically with 0.05% (w/v) biotin-conjugated polysaccharide aqueous solutions (sacran or HA) and then were cultured for 24h at 37°C. The medium of each culture was collected to quantify biotin-conjugated polysaccharides that had penetrated through the RHEEs. Each RHEE was cut into two pieces to provide for the quantification of biotin-conjugated polysaccharides remaining in the RHEE and for histological study. The culture media and the supernatants of homogenized RHEEs were used to determine biotin-conjugated polysaccharides in RHEEs using an ELISA method. The supernatants of RHEEs were prepared by the following procedure: RHEEs were homogenized in 500 μ L phosphate buffered saline (PBS) at 2700rpm for 10min with a μ T-12 bead crusher (Taitec Corp., Saitama, Japan) and were centrifuged at 15000rpm for 5min at 4°C. A competitive ELISA method was used for the quantification of biotin-conjugated polysaccharides as follows: Each culture medium or supernatant of RHEEs was mixed with an equal amount of streptavidin-HRP (1:1000) and was incubated at 37°C for 1h. The resulting mixture was used for the ELISA assay as a sample. To coat biotinylated bovine serum albumin (B-BSA)

on each well of an ELISA plate, the plate was incubated at 37°C for 1h after placing 150 μ L B-BSA in each well. After rinsing three times with 200 μ L PBS-T for 5min each, the plate was incubated with 150 μ L blocking solution at 37°C for 1h. Samples (100 μ L) were placed into wells and incubated at 37°C for 2h. After adding 100 μ L of the coloring solution (300 μ g/mL ABTS with 10 μ L H₂O₂), the plate was reacted at room temperature for 20min in the dark. The absorbance of the resulting solution at 405nm was measured using a microplate reader (Spectra Max Gemini, Molecular Devices, San Jose, CA, U.S.A.).

In the histological study, biotin-conjugated polysaccharides in RHEEs were visualized by staining frozen thin sections with DyLight650-labeled streptavidin, and nuclei were concomitantly stained with Hoechst33342. Fluorescence images were taken using a Fluid Cell Imaging Station (Thermo Fisher Scientific).

Cell Culture HaCaT keratinocytes were cultured in DMEM with 5% FBS at 37°C in a humidified atmosphere containing 5% CO₂.

Cell Damage HaCaT keratinocytes were cultured with SLS at various concentrations in the presence or absence of 0.02% (w/v) sacran or HA for 24h and 200 μ M α -tocopherol, which is a typical antioxidant, was used as a positive control. Cell viability was measured using the neutral red assay. Cells were further cultured in DMEM containing neutral red at a concentration of 33 μ g/mL for 2h. After washing with PBS, neutral red that was incorporated into living cells was extracted with a 30% MeOH aqueous solution under agitation. The absorbance of the resulting solution at 550nm was measured using a microplate reader. Cell viability is expressed as a percentage of absorbance against that of sham-treated cells.

Intracellular ROS HaCaT keratinocytes were cultured with SLS or IL-1 α in the presence or absence of sacran or HA for 24h. Cells were treated with 20 μ M H₂DCFDA in HBSS for 30min. After washing with HBSS, cells were cultured in DMEM containing various concentrations of detergents for 24h. After lysing the cells with 0.1% Triton X-100 in PBS, the fluorescence (Ex; 485nm, Em; 530nm) was measured with a fluorescence microplate reader (Spectra Max Gemini, Molecular Devices). Intracellular ROS levels are calculated as fluorescence intensity (F.I.) per μ g protein and are expressed as a fold change of the value of control cells. Protein concentrations were determined using a BCA-protein assay kit.

Membrane Fluidity Using the ESR Spin-Labeling Method Alterations in membrane fluidity of liposomes and HaCaT keratinocytes were estimated using an ESR spin-labeling method¹⁷⁾ with 5-DSA as an ESR spin-labeling agent.¹⁸⁾ Liposomes were prepared with lipids in which lecithin and cholesterol were mixed in a ratio of 2:1, using the Bangham method.¹⁹⁾ Liposomes and HaCaT keratinocytes were spin-labeled in the membrane region with 1mM 5-DSA and 10mM 5-DSA, respectively.

The order parameter (S) was calculated from the ESR spectra using the following equation:

$$S = (A_{\parallel} - A_{\perp}) / [A_{zz} - 1/2(A_{xx} + A_{yy})]$$

$$(A_{xx}: 0.63 \text{ mT } A_{yy}: 0.58 \text{ mT } A_{zz}: 3.36 \text{ mT})$$

ESR spectra were obtained using a RFR-30 spectrometer (Tokyo, Japan) at 37°C under the following conditions: modulation frequency, 100kHz; modulation amplitude width,

0.1 mT; scanning field, 338.1 ± 5 mT; receiver gain, 200; response time, 0.03; sweep time, 8 min; and output power, 4 mW.

Statistical Analysis All study data are expressed as means $\pm$ standard deviation (S.D.) Significant differences between experimental values were determined using the one-way ANOVA followed by Dunnett's test, and *p*-values of less than 0.05 are considered statistically significant.

RESULTS

Dynamics of Topically Applied Polysaccharides to Penetrate into RHEEs We examined the potential of the polysaccharides sacran and HA to penetrate through the stratum corneum (SC) using RHEEs cultured for 6 d and for 12 d as models having an immature SC or a mature SC, respectively. Hyper-fluorescence originating from sacran or from HA was localized at the surface of RHEEs cultured for 12 d (Fig. 1). The penetration of either polysaccharide through the RHEEs was not observed, and most of the polysaccharides stayed within the RHEEs (Fig. 1). On the other hand, fluorescence originating from sacran or HA was observed at the surface of RHEEs and also within RHEEs cultured for 6 d. In addition, approximately 50% of polysaccharides topically applied to the RHEEs were quantified in the culture medium. These results indicate the possibility that SC with an impaired barrier

function is penetrated even by these high molecular weight substances.

Protective Effects of Polysaccharides on Cell Damage Induced by SLS It is well known that SLS is a representative irritant, and often causes skin roughness. The effects of polysaccharides to alleviate the cytotoxicity induced by SLS at 50 and 100 μ g/mL were examined using HaCaT keratinocytes. Both polysaccharides, sacran and HA, alleviated the SLS-induced cell damage (Fig. 2). In addition, α -tocopherol, which is a typical antioxidant, also alleviated the SLS-induced cell damage. In order to clarify the relationship between cell damage and intracellular ROS levels in HaCaT keratinocytes, treatment with SLS at several concentrations was examined. It was found that the cell damage induced by SLS correlated significantly with intracellular ROS levels (Fig. 3).

Suppressive Effects of Polysaccharides on ROS Generation Stimulated by SLS or by IL-1 α The effects of polysaccharides on ROS generation by HaCaT keratinocytes induced by SLS or by IL-1 α were examined. HaCaT keratinocytes had elevated levels of intracellular ROS from 4.04 ± 0.22 F.I./ μ g protein to 17.92 ± 1.47 or 7.65 ± 0.95 F.I./ μ g protein (4- or 2-fold changes compared to the control) after culture in the presence of SLS at 50 μ g/mL or IL-1 α at 50 ng/mL, respectively (Fig. 4). Both polysaccharides attenuated the elevation of intracellular ROS levels stimulated by SLS and by IL-1 α . Sacran

Fig. 1. Penetration of Biotin-Conjugated Sacran in RHEEs

RHEEs were treated topically with or without 0.05% biotin-conjugated polysaccharide aqueous solutions (sacran or HA) as noted and then were cultured for 24 h at 37°C. The culture media were collected to quantify biotin-conjugated polysaccharides that penetrated through the RHEEs. (a) The histology of biotin-conjugated polysaccharides is shown as representative images (scale bars, 100 μ m). PBS was used as a control. (b) The penetration or retention of biotin-conjugated polysaccharides in RHEEs is expressed as a percentage of the amount of biotin-conjugated polysaccharide applied (*n*=4).

Fig. 2. Protective Effects of Polysaccharides against Cell Damage of HaCaT Keratinocytes Induced by SLS

HaCaT keratinocytes were cultured with SLS at various concentrations in the presence or absence of 0.02% polysaccharides (sacran or HA) and 200 µM α -tocopherol as noted for 24h. Cell viability was measured using the neutral red assay and is expressed as a percentage against the sham-treated cells. Dunnett's test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ ($n = 4$).

Fig. 3. Relationship between Cell Damage and ROS Generation in HaCaT Keratinocytes Induced by SLS

HaCaT keratinocytes were cultured with SLS at the concentrations noted (0, 6.25, 12.5, 25, 50 and 100 µg/mL) for 24h. Cell viability was then measured using the neutral red assay and is expressed as a percentage against the sham-treated cells. Intracellular ROS levels were measured using H_2DCFDA and are reported as F.I. per µg protein ($n = 5$).

showed a superior attenuation compared with HA.

Effects of Pretreatment with Sacran on SLS-Induced Cell Damage In order to determine the mechanism by which sacran protects against cell damage induced by SLS, the protective effects of sacran when used to pretreat cells in advance of exposure to SLS were examined. Pretreatment with sacran failed to protect against cell damage induced by SLS at various concentrations (Fig. 5).

Interaction between Sacran and the Lipid Bilayer Struc-

Fig. 4. Suppressive Effects of Polysaccharides on ROS Generation in HaCaT Keratinocytes Induced by SLS or IL-1 α

HaCaT keratinocytes were cultured with 50 µg/mL SLS or 50 ng/mL IL-1 α in the presence or absence of polysaccharides (sacran or HA) as noted for 24h. Intracellular ROS levels were measured using H_2DCFDA and are reported as F.I. per µg protein. Dunnett's test, ** $p < 0.01$, *** $p < 0.001$ ($n = 7$).

Fig. 5. Protective Effects of Sacran against Cell Damage of HaCaT Keratinocytes Induced by SLS

HaCaT keratinocytes were pretreated with or without 0.02% sacran for 24h after which they were cultured with SLS at various concentrations as noted. Cell viability was measured using the neutral red assay, and is expressed as a percentage against sham-treated cells. Dunnett's test, ns; not significant, *** $p < 0.001$ ($n = 5$).

Fig. 6. Changes of Order Parameters (S) of Liposomes and HaCaT Keratinocytes Treated with SLS Assessed Using ESR Spin-Labeling

The membrane fluidity of (a) liposomes and (b) HaCaT keratinocytes was estimated using an ESR spin-labeling method¹⁸⁾ with 5-DSA as an ESR spin-labeling agent. control (sham-treated liposomes and HaCaT keratinocytes), sacran (liposomes or HaCaT keratinocytes were treated with 0.02% sacran), SLS (liposomes or HaCaT keratinocytes were treated with 50 µg/mL SLS), pretreatment with sacran+SLS (liposomes or HaCaT keratinocytes pretreated with 0.02% sacran were exposed to 50 µg/mL SLS), sacran+SLS (liposomes or HaCaT keratinocytes were exposed to 50 µg/mL SLS and 0.02% sacran). Dunnett's test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ ($n = 5$).

ture In order to identify the potential interactions of sacran with cell membranes, we measured membrane fluidities of the lipid bilayer of liposomes prepared with lecithin and cholesterol, or HaCaT keratinocytes as order parameters using the ESR spin-labeling method (Fig. 6). The results indicate that sacran does not interact with the lipid membrane because sacran gave a constant value of order parameters (S) in liposomes and in HaCaT keratinocytes. On the other hand, since SLS gave lower values of S in both the lipid membranes of liposomes and of HaCaT keratinocytes, SLS increased the membrane fluidity due to its interactions with the lipid bilayer structure. However, the addition of sacran completely restored the decreases of S , which indicates that sacran interferes with the interaction between SLS and the lipid bilayers of liposomes and cell membranes.

DISCUSSION

In a previous human study, we reported that sacran improves skin conditions, both for healthy subjects and for patients with AD.^{9,10)} The SC parameters indicated that sacran modulates the terminal differentiation of the epidermis and alleviates the inflammatory symptoms of subjects who have impaired skin barrier functions. In addition, previous studies regarding the physico-chemical behaviors of sacran and composites of sacran and polyols, demonstrated their unique characteristics in which sacran or the composites with polyols formed water insoluble gel-like sheets that had protective effects against chemicals and interfered with their penetration.⁸⁾ Although those results suggested that the physico-chemical properties are responsible for the improvement of skin conditions of subjects with an impaired skin barrier, the

effects of sacran penetrating into the epidermis are also expected. Thus, in order to discuss the mechanism by which sacran improves skin conditions when it penetrates into the skin, we conducted the following examinations. Because sacran is a high molecular weight polysaccharide, it might be difficult to penetrate into the skin. Thus, the ability of sacran to penetrate into the skin was examined using RHEEs cultured for 6 d or for 12 d. Although approximately 50% of the sacran penetrated the immature SC of RHEEs cultured for 6 d, it did not penetrate the mature SC of RHEEs cultured for 12 d. HA, which is a typical polysaccharide used in cosmetics, also showed a similar behavior (Fig. 1). These results suggest that sacran and HA function in the epidermis due to their penetration through the SC with impaired barrier function.

SLS, a typical anionic surfactant, is widely formulated in detergents. In addition, it is well known that SLS at a high dosage causes skin irritation and is often used to induce roughened skin experimentally.^{12,13)} Thus, we examined whether polysaccharides prevented the cell damage induced by SLS. At first, to address the role of ROS on cell damage induced by SLS, we examined the relationship between cell viability and intracellular ROS levels and the effect of α -tocopherol to ameliorate the cell damage. Intracellular ROS levels correlated significantly with cell viability (Fig. 3) and α -tocopherol also showed a significant attenuation of SLS-induced cell damage. Based on those results, we demonstrated that ROS is responsible for the cell damage induced by SLS. On the other hand, sacran and HA also attenuated the cell damage induced by SLS (Fig. 2). In addition, sacran showed a superior attenuation compared with HA. Although sacran and HA do not possess any antioxidation properties, they reduced the cell damage. Thus, it seems that sacran and HA have a different mechanism to attenuate cell damage other than the scavenging of ROS.

A previous study reported that SLS enhances the generation of intracellular ROS through the stimulation of Ca^{2+} influx. In addition, IL-1 α also generates ROS through the activation of NADPH oxidase by IL-1R-associated kinase-1 signaling.²⁰⁾ As a biological parameter of dry skin, which is an early stage of rough skin, a higher ratio of IL-1RA and IL-1 α ²¹⁾ and a higher level of carbonylated proteins in the SC¹³⁾ have been reported. Protein carbonylation is initiated by aldehyde compounds synthesized by a peroxidation reaction with unsaturated lipids and ROS.²²⁾ In addition, it has been reported that the level of carbonylated proteins in the SC correlate well with the dysfunction of skin hydration.²¹⁾ Because subjects with impaired skin barrier function are characterized by low skin hydration, a high ratio of IL-1RA and IL-1 α and a high level of carbonylated proteins in the SC, we examined the suppressive effects of polysaccharides on ROS generation induced by SLS and IL-1 α . Although sacran and HA suppressed the intracellular ROS generation stimulated by SLS or by IL-1 α , sacran had a higher effect compared with HA (Fig. 4).

To sum up, these results indicate that polysaccharides attenuate the influence induced by extracellular stimuli, and that sacran has a superior efficacy compared with HA.

At the present, it is hard to understand the differences of sacran and HA on the attenuation effects. The molecular weight of sacran has been reported as 16 to 29 million Da, in contrast to HA, which is about 1 million Da. We do not yet know if the different effects could be due to the differences in

their molecular weights. To address that issue, further study is needed focusing on the relationship between the attenuation effect and the molecular weight.

Thus, we speculate that the action of polysaccharides to attenuate cell damage and reduce intracellular ROS levels is as follows; polysaccharides reduce the interaction of extracellular substances with cells due to shielding effects by adsorption on the cell membrane and/or trapping in their matrix. In general, we considered the possibility that polysaccharides interact with cell membranes due to their adsorption through lectins. Therefore, we considered the possibility that sacran interferes with interactions between cells and extracellular stimuli as a shield by adsorption on the cell surface. In order to test that hypothesis, the following experiments were conducted. To identify the adsorption of sacran on the cell membrane, we examined whether cells pretreated with sacran retain their function to alleviate cell damage induced by SLS. However, cells pretreated with sacran completely lost that alleviative function (Fig. 5). The results of sacran pretreatment on membrane fluidity in liposomes and cells suggest that sacran is not adsorbed on the cell surface, because pretreatment with sacran failed to abolish the influence of SLS. In addition, sacran did not influence the membrane fluidity of lipid bilayers in liposomes and in cell membranes (Fig. 6).

These results ruled out the possibility of the adsorption of sacran on the cell membrane. In order to investigate other possibilities, we measured the influence of SLS on membrane fluidity in the presence of sacran. Sacran restored the alteration of membrane fluidity caused by SLS (Fig. 6). The sum of those results indicates that sacran traps substances such as SLS and IL-1 α in its matrix, which results in the attenuation of SLS-induced cell damage and the IL-1 α induced ROS elevation.

Regarding the mechanism of the trapping effect of sacran against SLS or IL-1 α , we currently do not understand that. Sacran has been identified as an anionic polysaccharide that contains sulfated or carboxylated sugar. In addition, HA also is an anionic polysaccharide. On the other hand, SLS is a typical anionic surfactant. Although SLS is known to interact with the cationic biopolymer chitosan,²³⁾ it is generally thought that it would be difficult to make an ion complex with SLS and sacran or HA.

In our previous study, we reported that sacran is able to emulsify squalene due to the retention of squalene in its own matrix. Those facts suggest that polysaccharides such as sacran might have a hydrophobic pocket. Regarding the mechanism of trapping, we will continue to study that and will report it elsewhere.

The sum of these results suggest a possible mechanism for the alleviation function of sacran against extracellular stimuli as follows: sacran shows its alleviation function by interfering with interactions between extracellular stimuli and cells due to the trapping of stimuli in its own matrix.

CONCLUSION

As one of the mechanisms to improve skin conditions caused by impaired barrier functions, we demonstrated that sacran interferes with the attack of extracellular stimuli on cells due to its trapping effect even into the epidermis.

Conflict of Interest The authors declare no conflict of interest.

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