

Effect of Emollients Containing Vegetable-Derived *Lactobacillus* in the Treatment of Atopic Dermatitis Symptoms: Split-Body Clinical Trial

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Background: Atopic dermatitis (AD) patients suffer from xerosis. Proper skin care, including the use of emollients, may help improve xerosis and minimize disease exacerbation. *Lactobacillus sakei* probio 65, isolated from the Korean vegetable-based product kimchi, can decrease interleukin 4 and immunoglobulin E levels and inhibit *Staphylococcus aureus*. Moreover, it has reportedly shown positive dermatological effects in both animal and clinical studies. **Objective:** To compare the effects of an emollient that contains *Lactobacillus* (treated) with a normal emollient (control) on AD. **Methods:** This double-blind, randomized, split-body clinical trial involved 28 patients with AD. The patients applied the *Lactobacillus*-containing emollient on one side of their body and the control emollient on the other side twice daily for 4 weeks. Trans-epidermal water loss (TEWL) and skin capacitance were evaluated and investigator global assessment and the visual analogue scale (VAS) were administered on weeks 0, 1, 2, and 4. **Results:** The treated sides had significantly lower TEWL and VAS values and significantly higher skin capacitance values over time than the control sides. **Conclusion:** Topical application of *Lactobacillus*-containing emollients may improve the skin permeability of patients with AD. (**Ann Dermatol** 26(2) 150 ~

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-Keywords-

Atopic dermatitis, Emollients, *Lactobacillus*

INTRODUCTION

Atopic dermatitis (AD) is generally characterized by pruritic skin lesions and chronic eczematous lesions. It may occur in people of any age, but disease onset is more common in preschoolers¹. The management of AD requires a multipronged approach that includes repair of barrier function and general skin care, use of topical or systemic agents, and the identification and elimination of precipitating or exacerbating factors². Hydration of the skin is particularly important because dry skin gives rise to microfissures and cracks that allow the entry of pathogens, antigens, and irritants, thereby exacerbating the disease³. The regular use of emollients has many beneficial effects in AD. It improves the appearance and symptoms of dry skin³ and may reduce the need for topical corticosteroids by approximately 50%⁴. A study found that emollients enhance the response to treatment with topical corticosteroids⁵. In addition, a pilot study showed that emollients can prevent the development of AD in high-risk infants⁶. Indeed, some patients only use emollients due to concerns about the side effects of topical corticosteroids⁷. Several studies suggest that incorporating *Lactobacillus* may improve the performance of emollients in AD. In an animal AD model study, topical application of medicinal herbs fermented with *Lactobacillus plantarum* seemed to have a better therapeutic effect than the control and oral admini-

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stration groups⁸. In addition, Segawa et al.⁹ reported that intrarectal administration of *Lactobacillus*-derived polyphosphate enhances the epithelial barrier function in mouse small intestine.

L. sakei probio 65, the strain used in this study, was isolated from kimchi, which is a traditional fermented Korean food that is believed to reduce the risk of chronic diseases. A range of bacteria are involved in the kimchi fermentation process, with *Lactobacillus* being one of the major species. Of the various strains of this bacterium, *L. sakei* probio 65, exhibited the most potent inhibitory activity against *Staphylococcus aureus* growth in preliminary experiments. It also induced immunological and clinical improvements in an animal study and a clinical study^{10,11}. The present clinical trial was performed to determine whether the efficacy of emollients in AD could be improved by incorporating *L. sakei*.

MATERIALS AND METHODS

In the present study, *L. sakei* probio 65-containing emollient was tested for safety as a topical medication in a rabbit model, after which patients with AD were recruited and asked to apply the *L. sakei* probio 65-containing emollient to one randomly selected side of the body and a normal emollient (control) to the other side. The clinical outcome and electrical skin barrier functions of each side were then evaluated. The control and *L. sakei* probio 65-containing emollients were identical except for the presence of *L. sakei* probio 65 in the latter.

Animal study

The rabbits were divided into three groups. The control group received normal saline, Group 1 received *L. sakei* probio 65 extract, and Group 2 received an *L. sakei* probio 65-containing emollient. This study was approved by the Chungnam National University Institutional Animal Care and Use Committee of Korea (CNUCOM-2007-017).

1) Eye stimulation test

The eyes of the Group 1 rabbits received 100 μ l of the liquid extract while the eyes of the Group 2 rabbits received 100 mg of the *L. sakei* probio 65 extract-containing emollient, from which the test compound was washed out with normal saline after 5 seconds.

2) Skin stimulation test

The flanks of the rabbits were shaved and one day after Groups 1 and 2 respectively received 100 μ l of the extract and 100 mg of the emollient via application with a cotton swab. The responses were evaluated after 24, 48,

and 72 hours.

Clinical study

1) Study design and patients

The clinical trial was performed between the end of June and the beginning of October 2011. The subjects were followed-up until November 2011. All subjects were recruited from the Dermatology Clinics of Chungnam National University Hospital in Daejeon, Korea. The patients or parents/guardians were provided with written informed consent before study entry. This study was approved by the Chungnam National University Hospital Institutional Review Board (IRB no. 1106-40). The 30 patients who participated in the study had been diagnosed with bilateral AD for at least 6 months according to Hanifin's criteria¹². The mean age of the patients was 14.2 years (range 3~37 years), and there were 20 males and 10 females. The AD (SCORing atopic dermatitis, SCORAD) total score of the patients ranged from 13.5 to 50.3 (mean 30.4). Patients who were treated with cyclosporine or systemic corticosteroids 4 weeks before the study were excluded. Continuing use of antihistamines and topical steroids was permitted. At baseline visit, one side of the body of each patient was randomly assigned to receive the *L. sakei* probio 65-containing emollient, while the other side was treated with the control emollient. The emollients were both applied twice daily.

2) Outcome assessments

Clinical visits were scheduled at baseline and after weeks 1, 2, and 4. The clinical outcome was evaluated by using clinical and objective parameters. Clinical parameters were assessed using the investigator's global assessment (IGA) and the visual analogue scale (VAS) for pruritus. The IGA of both sides of the body was graded by a dermatologist on a five-point scale (0, clear; 1, nearly clear; 2, mild; 3, moderate; 4, severe). The VAS assessments were performed by the patients themselves. Each patient was asked to score the severity of their pruritus subjectively on a scale of 0 to 10. Objective parameters included trans-epidermal water loss (TEWL) and skin capacitance. The TEWL of both sides was measured by using a Tewameter TM210 (CK electronic, Cologne, Germany). The skin capacitance was assessed using a Corneometer CM825 (CK electronic). Each objective parameter was measured three times at 23°C and at a relative humidity of 54% to 56%, after which the average measurement was calculated.

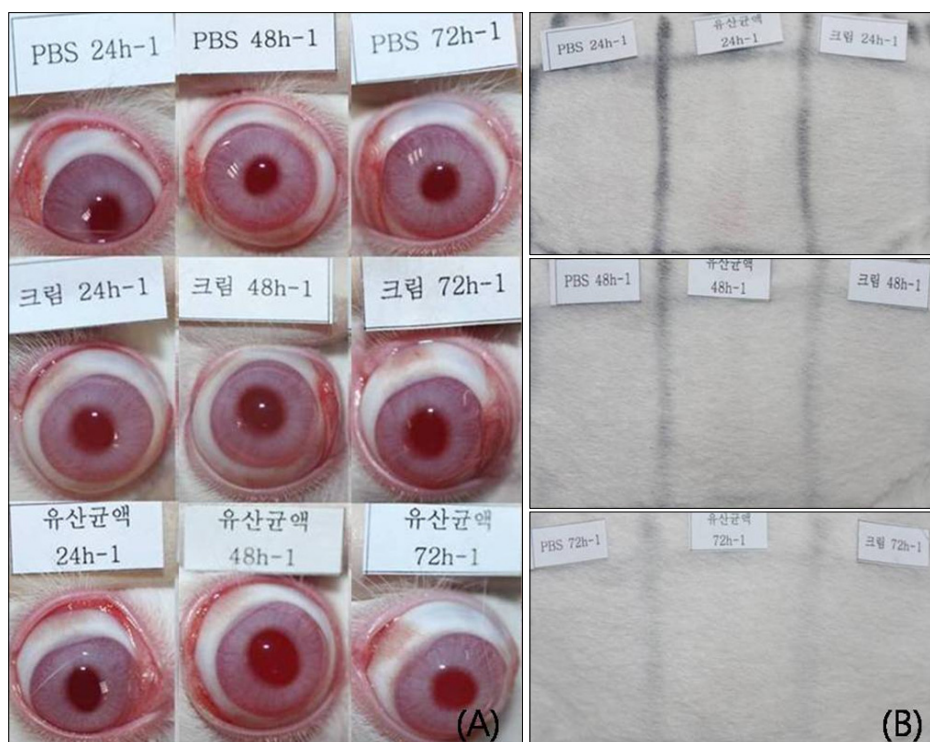


Fig. 1. Three groups showed no responses in eye stimulation test (top: PBS, middle: *Lactobacillus sakei* probio 65 extract containing emollient, bottom: *L. sakei* probio 65 extract) (A) and skin stimulation test (left: PBS, middle: *L. sakei* probio 65 extract, right: *L. sakei* probio 65 extract containing emollient) (B). PBS: phosphate buffer saline.

3) Statistical analysis

The baseline patient data were analyzed by using Student's t-test. The data of the treated side of the body were compared to those of the control side by using a repeated measure analysis of variance. IBM SPSS Statistics 19.0 software (IBM Co., Armonk, NY, USA) was used for statistical analyses. *p*-values of <0.05 were considered to be statistically significant.

RESULTS

Animal study

Fig. 1A and B show the eye stimulation and skin stimulation test results, respectively. None of the three groups showed a response 24, 48, and 72 hours after being exposed to the control or test agent.

Clinical study

Of the 30 subjects who were enrolled, two were lost to follow-up over the 4-week study period.

1) Baseline characteristics

The baseline clinical data of the subjects are summarized in Table 1. The treatment and control sides did not differ significantly in terms of baseline disease severity. As a result of random selection of the treatment side, the test

Table 1. Baseline data (IGA, VAS, TEWL, skin capacitance) on control and treatment sides

Baseline data	Treatment side (n=28)	Control side (n=28)	<i>p</i> -value
IGA	2.75 ± 0.70	2.71 ± 0.66	0.762
VAS	6.53 ± 1.77	6.50 ± 1.75	0.876
TEWL	37.10 ± 9.30	34.30 ± 8.40	0.479
Skin capacitance	27.40 ± 10.80	28.90 ± 10.00	0.639

Values are presented as mean ± standard deviation. IGA: investigator's global assessment, VAS: visual analogue scale, TEWL: trans-epidermal water loss.

emollient was applied to the right antecubital side in 10 subjects, the left antecubital side in 9 patients, the right popliteal side in 4 patients, and the left popliteal side in 5 patients.

2) Investigator's global assessment

Fig. 2 shows digital photographs of cases where substantial improvement was observed after the *L. sakei* probio 65-containing emollient was applied. Fig. 3A shows the IGA scores during the 4-week study period. None of the treatment sides were worse than their control sides, and in three cases, the treatment side had a better result than the control side. The mean difference between the IGA values of the treatment and control sides increased substantially from $-0.04 ± 0.19$ at the baseline

visit to -0.04 ± 0.43 at week 1, 0.14 ± 0.59 at week 2, and 0.18 ± 0.61 at week 4. However, these differences were not statistically significant ($p=0.366$).

3) Visual analogue scale of pruritus

The VAS scores during the 4 week study period are shown

in Fig. 3B. The mean VAS scores of both the treatment and control sides decreased during weeks 1, 2, and 4, and the mean difference between the VAS values of the treatment and control sides increased significantly from -0.04 ± 0.19 at the baseline visit to -0.04 ± 0.43 at week 1, 0.39 ± 0.79 at week 2, and 0.57 ± 0.96 at week 4 ($p=$



Fig. 2. Effect of emollients containing *Lactobacillus* in atopic dermatitis patients at baseline (A) and after 4 weeks (B).

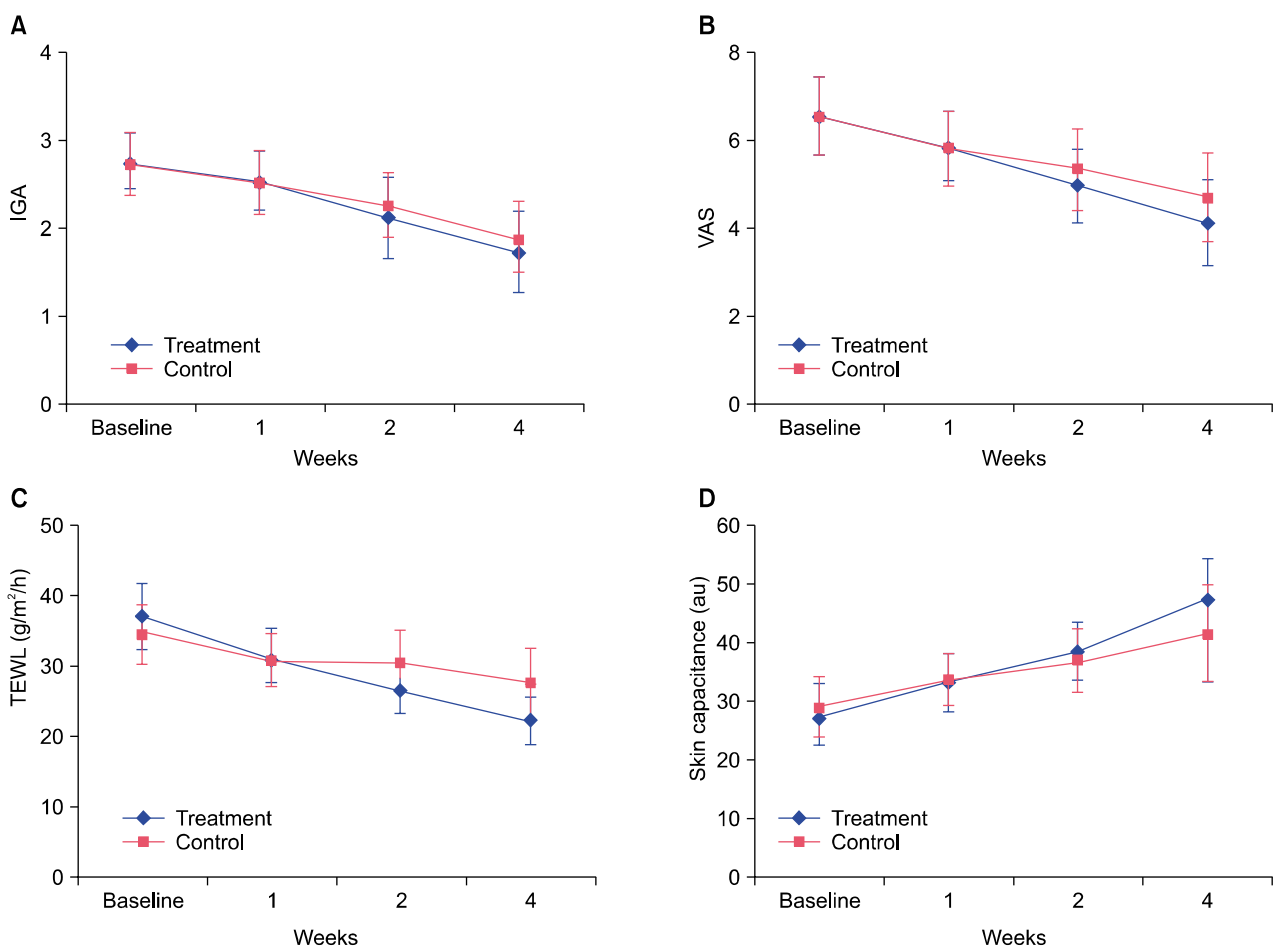


Fig. 3. Effect of emollients containing *Lactobacillus* assessed from IGA (A), VAS of pruritus (B), TEWL scores (C), and skin capacitance values of control and treatment sides (D). IGA: investigator's global assessment, VAS: visual analogue scale, TEWL: trans-epidermal water loss.

0.006). This suggests that the treatment side improved faster than the control side.

4) Trans-epidermal water loss

The TEWL data during the 4 week study period are shown in Fig. 3C. The mean TEWL of both the treatment and control sides decreased during weeks 1, 2, and 4, and the mean difference between the TEWL values of the treatment and control sides increased significantly from -2.74 ± 4.19 at the baseline visit to -0.51 ± 4.64 at week 1, 3.99 ± 7.05 at week 2, and 5.59 ± 9.75 at week 4 ($p = 0.007$). This suggests that the TEWL of the treated side improved faster than that of the control side.

5) Skin capacitance

Skin capacitance during the 4 week study period is shown in Fig. 3D. The mean skin capacitance of both the treatment and control sides increased at weeks 1, 2, and 4, and the mean difference between the skin capacitance values of the treatment and control sides increased significantly from -1.45 ± 4.10 at the baseline visit to -0.89 ± 5.53 at week 1, 1.75 ± 4.31 at week 2, and 5.95 ± 7.13 at week 4 ($p = 0.001$). This suggests that the treatment side developed better skin capacitance than the control side.

6) Adverse effects

The *L. sakei* probio 65-containing emollient was generally well tolerated. No significant adverse effects were encountered during the study, although three patients experienced mild application site reactions such as burning and stinging, which resolved within 3 days.

DISCUSSION

This study is the first randomized split-body controlled trial of *Lactobacillus* extract for the treatment of AD. No irritation was observed from the use of the extract in the animal study. The treated sides of the body showed no significant adverse effects when compared to the control sides in the clinical study. The TEWL, VAS, and skin capacitance values of the treated sides were significantly improved relative to the values of the control sides. However, the IGA values of the treated sides did not differ significantly from those of the control sides. This result can be probably explained by the short study period and the use of only five IGA grades in the scoring. Overall, the results suggest that the *L. sakei* probio 65-containing emollient is safe and beneficial in patients with AD.

Recently, numerous studies have suggested that the ability of *Lactobacillus* to inhibit allergic reactions may relate to its ability to improve the Th1/Th2 balance^{13,14}. Moreover,

Lactobacillus was shown to inhibit *S. aureus* growth and to improve the epithelial barrier function^{9,15,16}. However, a study on the *ex-vivo* immunomodulatory effects of probiotics in children with AD returned inconclusive results¹⁷. Most of the studies on *Lactobacillus* used the *L. rhamnosus* strain to evaluate the clinical and immunological responses. However, we chose *L. sakei* probio 65 as it was used in two studies previously. Park et al.¹⁰ described an animal study involving the oral administration of *L. sakei* probio 65 in which the treated group exhibited better clinical improvement and lower interleukin (IL)-4 and immunoglobulin (Ig)-E levels than the control group. In addition, a 12-week, double-blind, placebo-controlled trial showed that oral administration of *L. sakei* probio 65 resulted in clinical improvement of patients with AD¹¹. In the present study, a *L. sakei* probio 65-containing emollient was applied topically in patients with AD. Studies on topical *Lactobacillus* application in patients with AD are rare. Joo et al.⁸ conducted a study using an AD rat model where medicinal herbs fermented with *L. plantarum* were administered topically. They found that topical application resulted in better histological results than oral administration. Two other studies of topical application of *Lactobacillus* in burn victims showed that this treatment improved tissue repair and inhibition of bacteria^{18,19}. Thus, the few studies on the topical use of *Lactobacillus* support the observations of the present study.

L. sakei probio 65-containing emollients may have some advantages over other emollients in the treatment of patients with AD. First, *L. sakei* can inhibit the growth of *S. aureus*, a known immunological trigger¹ and cause of common complications (such as impetigo)²⁰. Second, it has immunomodulatory effects that are likely to decrease the IL-4- and Ig-E-induced acute phase of AD^{17,18}, although additional studies are needed to confirm that topical application of such an emollient has these effects as well. Third, *Lactobacillus*-derived polyphosphate improved the ability of the intestinal epithelium to defend itself from injurious stimulants^{9,15,16}. Thus, *Lactobacillus*-derived polyphosphate may have similar effects on the skin epithelium. These potential advantages of *L. sakei* probio 65-containing emollients may explain why topical application of the emollient improved the skin barrier and resulted in the improvement of symptoms in AD patients in the present study.

The limitations of the present study are that it was a single center study involving a relatively small group of subjects who were treated for a short period. Moreover, there was concomitant treatment with topical steroids in most cases. It is unclear whether the use of topical steroids affected

the efficacy of the *L. sakei* probio 65-containing emollient. However, since the patients in the present study used topical steroids on both sides, the observed effects of the *L. sakei* probio 65-containing emollient remain clinically meaningful. However, the topical steroid-independent effects of *L. sakei* probio 65 should be evaluated in the future.

In summary, this double-blind, randomized, split-body study, in which many variables were controlled, suggests that *L. sakei* probio 65-containing emollients may be beneficial and safe for use in patients with AD who are using a topical steroid cream.

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