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Application of a Synbio-Glucan Functional Spray for Canine Atopic Dermatitis

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Abstract Atopic dermatitis (AD) is a common skin disease in animals and several therapeutic trials with various drugs have been conducted for more effective management of AD. However, these trials have not been able to properly address all the aspects of AD management because of the lack of good efficacy or due to significant side effects of the drugs being tested. Synbio-glucan functional spray is a functional skin spray using Synbio-glucan composed of β-glucan and probiotics. We designed a functional spray composed of Synbio-glucan (patent application number:10-1805863), distilled water, glycerin, solubilizer, and 40% alcohol. We tested the efficacy and safety of the functional spray on six dogs with AD. The trial was conducted with the consent of the caregivers. The spray was applied to the skin lesions, including the trunk, axillae, inguinal region, or periocular areas, thrice a day for 30 days. To evaluate the efficacy of this functional spray, we assessed the pruritus visual analog scale (PVAS) and the canine atopic dermatitis extent and severity index (CADESI)-4. At the end of one month, the results clinical scores after functional spray treatment showed a significant decrease in the PVAS ($p = 0.03$) and CADESI-4 ($p = 0.03$) in all the subject dogs with AD. This study thus confirmed that the Synbio-glucan functional spray is efficacious and safe for the treatment of AD in dogs.

Key words atopic dermatitis, β-glucan, dog, probiotics.

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Introduction

Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by pruritus, dry skin, erythema, edema, or lichenification and is a very common condition seen in humans and animals, especially dogs and cats (10,14). Although the causes of AD have not yet been fully understood, it is known that several factors, including environmental and genetic factors, and skin immune abnormalities, are involved in the pathogenesis. It has been thought that pruritus occurs due to dermal hypersensitivity to external allergens. Itch-scratch leads damage to the skin barrier, facilitating secondary penetrations and infections of skin pathogens such as bacteria and fungi, which further exacerbating pruritus (10,41,50,56). It has recently been accepted that AD probably results from an interaction between the host immune system and environmental triggers (32,41). As the awareness of the similarities between human and animal AD has increased, many comparative studies on pathological mechanisms and the development of new therapeutic agents are being conducted actively. In a trend similar to humans, AD is becoming increasingly common in dogs (37). According to an earlier study that evaluated the prevalence of several disorders in 192 canine breeds in the UK, more than 36% of the disorders were related to the skin, ears, and coat. Atopic allergic skin diseases accounted for 8.7-21.6% of disorders, and this proportion is gradually increasing (18,56).

The two primary goals of AD treatment are the control of acute flares and long-term management and, in this context, several drugs and therapies have been evaluated for better control of the disease. The mechanisms of action of therapeutic drugs specific to AD mainly include the following: maintenance of the skin barrier by alleviating pruritus and moisturizing the skin, regulation of the microorganisms present on the skin surface, and regulation of dermal immune response. Drugs used for AD management in humans have also been suggested for the treatment of dogs, but insufficient efficacy and the presence of side effects have limited their use (37,41,50). Therefore, there was a need to develop a therapeutic agent with better efficacy and fewer side effects. In an earlier study, we confirmed the efficacy of Synbio-glucan composed of β-glucan and probiotics on an AD mouse model (25). This study evaluated the efficacy of a functional spray composed of Synbio-glucan, distilled water, glycerin, solubilizer, and 40% alcohol for the effective management of AD in dog patients.

Materials and Methods

Case selection and inclusion criteria

Six dogs diagnosed with AD at the Kangwon National University Veterinary Hospital were included in the study between 2019 to 2020 based on Favrot's criteria (41). The trials were conducted with the consent of the caregivers and the Kangwon National University Institutional Care and Animal Use Committee approved all the protocols and guidelines for animal care (Approval No. KW-200109-1). All patients underwent physical examination, blood tests, imaging tests, urinalysis, and hormone tests. Patients with common skin infections, skin tumors, and hormonal skin diseases were excluded from the study. Only patients with chronic pruritus for several weeks and a pruritus visual analog scale (PVAS) score of 5 or higher were included in the study. Table 1 shows the baseline characteristics of the patients.

Production of functional spray

Synbio-glucan is a mixture of β-glucan, oat lipids, oat peptides, oat flavonoids (phenolic structure), avenanthramides, tocopherol (Vitamin E), and sphingomyelinase. The oats were preprocessed by heating them at over 80°C and fermented with probiotics (*Lactobacillus plantarum*, *Bifidobacterium longum*, and *Pediococcus pentosaceus*) to produce Synbio-glucan (patent application number:10-1805863) (25). To develop 100 mL of functional spray, Synbio-glucan (30 mL), distilled water (51.4 mL), glycerin (2 mL), solubilizer (0.6 mL), and 40% alcohol (16 mL) were mixed using an agitator and then stored in opaque bottles at 5˚C in the refrigerator.

Treatment with functional spray

The monotherapy of Synbio-glucan functional spray was performed three times a day for 30 days on skin lesion areas, including the trunk, axillae, inguinal region, muzzle, periocular areas, pinnae, the flexural surface of the elbow,

Table 1. The characteristics of dogs with atopic dermatitis

Dogs	Age (year)	Sex	Breed	Treatment history
1	2	Castrated male	Shiba Inu	Lokivetmab*
2	3	Spayed female	Maltese	·
3	3	Intact male	French Bulldog	·
4	6	Intact male	Shih Tzu	·
5	5	Intact male	Yorkshire Terrier	·
6	4	Intact male	Poodle	·

The mean age was 3.8 years. Only one dog had a history of treatment for atopic dermatitis.

*30 mg/dog subcutaneously injected monthly.

and interdigital areas. The spraying was applied at a distance of approximately 5 cm from the skin lesion site. Other skin treatment regimens like the topical or systemic therapy were not included.

Clinical assessment

The efficacy of the functional spray was evaluated clinically using previously known methods, to effectively quantify the severity of the AD symptoms before and after treatment (11,17,43,48). The degree of pruritus-related behaviors, such as scratching, licking, biting, and rubbing, was evaluated by the pruritus visual analog scale (PVAS) according to the following scores by each caregiver: 0 = normal; 1-2 = rarely itching (occasional episodes); 3-4 = mild pruritus (semi-frequent episodes); 5-6 = moderate pruritus (frequent episodes); 7-8 = moderate to severe pruritus (prolonged episodes); 9-10 = severe pruritus (extremely continuous itching) (17,48). The degree of skin lesions was scored with canine atopic dermatitis extent and severity index-4 (CADESI-4) designed by the International Committee on Allergic Diseases of Animals (ICADA) (43). The index evaluated twenty different body parts. The skin lesions of erythema, lichenification, and excoriation/alopecia were scored as normal 0, mild 1, moderate 2, and severe 3. The sum of the evaluated values was expressed as mild (10-34), moderate (35-59), and severe (more than 60). With 20 sites, three lesions, and four severity scores (0-3), the maximum score was 180 ($20 \times 3 \times 3 = 180$) and the minimum score was 0 (11). The evaluations were made by one assessor for consistency. After treatment, subsequent worsening clinical symptoms such as skin irritation, redness, severe scale, and pruritus were considered side effects.

Statistical analysis

The statistical analyses of all the data were performed using the GraphPad Prism (ver. 5.01; GraphPad, USA) statistical analysis software. The values shown represent the means of the experiments performed for each experimental group. Differences between the means were identified by performing Wilcoxon signed rank tests. A $p < 0.05$ was considered to indicate significance.

Results

A total of six dogs presented with skin problems at the time of referral. The clinical abnormalities observed were typical skin lesions of canine AD; systemic and extensive pruritus and erythema, lichenification, focal or general hair loss, and scratches in the neck, elbow joint, interphalangeal region, and around the eyes. The patients exhibited remission of skin lesions approximately one month after the treatment with the Synbio-glucan functional spray (Fig. 1). Dog 1 was treated with additional lokivetmab (30 mg/dog subcutaneously injected monthly) to control severe itching and showed relief of recurrent pyoderma and alopecia.

Fig. 1. Clinical improvement was confirmed after the administration of the functional spray. (A) Before treatment, (B) 30 days after treatment.

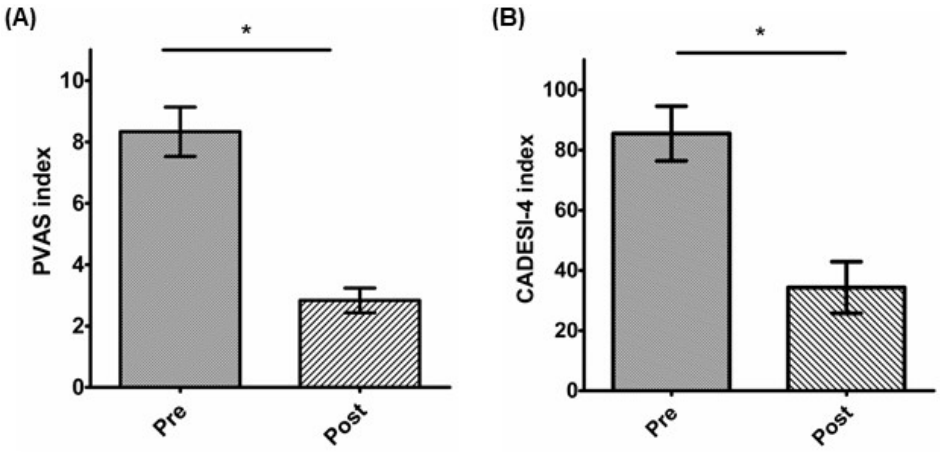

Fig. 2. The clinical efficacy of the functional spray with Synbio-glucan on canine atopic dermatitis. The scores before and 30 days after treatment were significantly decreased on the pruritus visual analog scale (PVAS). (A) (*p = 0.0355) and CADESI-4. (B) (*p = 0.0355) (Wilcoxon signed rank test).

The results of the statistical analysis of the clinical scores after functional spray treatment showed a significant decrease in the PVAS scores before the treatment and one month subsequently (Wilcoxon signed rank test) (p = 0.0355) (Fig. 2A). Also, according to the CADESI-4 scores, there was a statistically significant difference in the status before and one month after the functional spray application (p = 0.0355) (Fig. 2B). The significant decrease in the PVAS and CADESI-4 scores denoted a significant improvement in skin symptoms. No side effects were seen in patients related to the use of the functional spray.

Discussion

The efficacy of the functional spray was evaluated clinically using previously known methods, to effectively quantify the severity of the AD symptoms before and after treatment (11,17,43,48). In this study, we found that the Synbio-glucan functional spray treatment had remarkable clinical efficacy on canine AD. After the treatment, all the patients (6 out of 6 dogs) demonstrated a significant decrease in both the PVAS and CADESI-4 scores. Additionally, based on the feedback of the caregivers, the overall quality of life of the patients improved, as demonstrated by indicators such as sleep and appetite.

Topical treatment is the primary therapy not only in mild but also in severe cases of AD in humans. In canines, topical treatment includes decreasing the amount of allergen trapped in the coat, improving the skin barrier function with appropriate moisturizing agents, delivering anti-inflammatory agents, and resolving secondary infections (37,41). Various pharmacological agents to control AD have been developed, but they have several limitations along with their advantages. Glucocorticoids, tacrolimus (57,58), essential fatty acids, anti-histamines (37,42), allergen-specific immunotherapy (46), and monoclonal antibody, lokivetmab (40) are popular treatments, but previous studies have reported their side effects and limitations.

Thus, recent studies have begun to focus on therapeutic agents that can be obtained from nature, one of them being biologically active polysaccharides (BAPs) like β-glucan, β-fructans, or chitin, and probiotics. β-glucan is present in the cell walls of yeast, mushrooms, cereals or plants, algae, and some bacteria. Some earlier studies on β-glucan evaluated its efficacy in treating or preventing AD, but the sources were algae and yeast and not cereals such as oats, wheat, or corn (20,22,51). Oats have been suggested as a source of β-glucan as they are a rich reservoir of natural nutrients and biologically active substances (30). β-glucan from bacteria, fungi, and yeast have been shown to induce the proliferation of regulatory T cells in inflammatory bowel disease, periodontitis, and autoimmune encephalomyelitis mice models (5,31,34). Also, previous studies have confirmed that these effects of β-glucan can be helpful in the treatment and prevention of allergic diseases in humans (1,19,44). Some areas where β-glucan has found therapeutic use include the prevention of bacterial infections (8), wound healing (45), relief from allergic skin diseases (27), and cosmetics for sensitive and irritated skin (55). In another study β-glucan cream developed from mushrooms decreased the duration of the intensity of AD flares and relieved the intensity of pruritus within a few days of regular application in human AD (20). In this study, itch-scratch control with topical oat β-glucan treatment would be helpful in AD management.

Nowadays, there are many probiotic products on the market, and they are widely used in humans. To produce the Synbio-glucan used in this study, β-glucan derived from oats was fermented with probiotics comprising *Lactobacil-*

lus plantarum, *Bifidobacterium longum*, and *Pediococcus pentosaceus*. Probiotics are defined as live microorganisms, that, when administered in adequate amounts, confer a health benefit to the host (47). Thus, probiotic bacterial therapy has been suggested as having great potential in the treatment and prevention of skin conditions. Due to this skin health-promoting effect, several strains of probiotics are used as supplements in immune-mediated skin diseases (6,61). Many experimental studies have shown that the probiotics influenced on epithelial cells and on immune cells in the intestine, resulting in a lowering of allergic reactions. Also, due to their benefits associated with skin health and AD-related symptoms, the topical application of probiotics is an emerging approach in dermatology (3,6,9,15,26,39).

Among the several strains of probiotics, *Lactobacillus plantarum* is commonly found in many fermented food products such as yogurt and kimchi. Its immune-related effects, antimicrobial, anti-biofilm, antioxidant, anti-inflammatory, and vitamin B2-producing properties are already well known (16,24,33,38). Experimental studies involving the oral administration of *Lactobacillus plantarum* and β-1, 3/1, 6-glucan to animal models of AD have been conducted. These studies have confirmed that *Lactobacillus plantarum* alleviated AD through a significant decrease in the mRNA levels of Th2 and Th17 cell transcription factors while increasing the transcription factors of Th1 and regulatory T (Treg) cells, galactin-9, and filaggrin. This is indicative of enhanced immunomodulatory properties (21,23).

Bifidobacterium is a normal bacterium present in the intestines of animals and is used widely as an immune regulatory supplement. A representative probiotic, *Bifidobacterium longum*, has been studied as an immunomodulatory agent in adult patients with AD (60). Deficiencies in riboflavin can lead to skin disorders in dogs and cats as well as humans (29,54). Two *bifidobacteria* strains (*B. infantis* and *B. longum*) have been reported to increase the levels of riboflavin. A clinical trial involving the oral administration of *Bifidobacterium* longum in dogs with AD reported that the probiotic was not very effective in reducing pruritus, but was effective in improving skin lesions (35). Researchers suggest a direct and an indirect mechanism of action of *Bifidobacterium* in AD: direct action by inhibiting the release of neuro-mediators and decreasing neurogenic inflammation, frequently associated with sensitive skin symptoms, and indirect action by improving the skin barrier function and protecting neurons from external stimuli (13).

The alleviative effects of *Pediococcus pentosaceus* on AD have yet to be well studied (25). Still, one study of allergic contact dermatitis induced by dinitrofluorobenzene found that inflammatory responses and contact dermatitis were alleviated by significantly inhibiting LPS-stimulated phosphorylation and NF-κB (28). In a paper on probiotics in AD, the authors state that probiotic treatment is a beneficial approach for patients, with no side effects and high efficacy, and is thus potentially comparable to the conventional methods of treatments (26).

Inflammation which includes allergic inflammation causes the dysfunction of the skin barrier and the weakened barrier increases exposure to allergens and antigens (37). Therefore, maintaining the skin barrier is important for successful long-term AD management. Most compromised skin barriers are characterized by abnormally dry, itchy, or cracked skin, and are susceptible to further infection and irritation (50). Moisturizers are an essential cosmetic component for maintaining skin barriers in healthy and atopic skin (7,49). β-glucan found in oats is composed of β-(1,3) linkages and a small number of β-(1,4) linkages (4). Plant-derived β-glucan, from plants such as oats, is insoluble in water. Therefore to overcome this, a solubulizer and glycerin are added in the medicinal and cosmetic industry (59). Glycerin, as a natural moisturizer, has long been known for its topical benefits. It is commonly added to various cosmetics because of its moisturizing capacity (12). Oil-in-water emollients are known to complement the skin barrier function of normal and atopic skin by improving transepidermal water loss and skin corneometer values (which indicates the degree of hydration) (36,50). The proportion of glycerin in our functional spray was 2% and a safe use concentration was set based on human cosmetic sprays (2). The ratio of ingredients in the functional spray was set arbitrarily. The proportion of glycerin referred to the existing human literature and was set arbitrarily within the range of ratio used. Future studies on more detailed component ratios would be needed. This moisturizing property of glycerin and β-glucan make them an effective skin barrier protective supplement. Also, for long-term AD management, β-glucan has been confirmed to be clinically effective as a good steroid-sparing medication in mild to severe AD (7,20).

In this study, there were no side effects seen in patients related to the use of the functional spray. There were few reports of side effects or skin allergies after β-glucan administration, but those negative effects were either confirmed to be caused by other drugs (e.g., non-steroidal anti-inflammatory drugs) or were very minor (52,53). The therapeutic advantages discussed above are expected to make the Synbio-glucan functional spray an effective topical remedy for canine AD. The β-glucan preparation refined with probiotics and moisturizers allows for better skin absorption. Moreover, the functional spray formulation has a ready market because it

meets the needs of canine AD patients and their caregivers.

In this study, we designed a functional skin spray using Synbio-glucan (patent application number:10-1805863) and applied it to the skin lesions of dogs with AD. The efficacy was evaluated and the clinical status before and after the application was comparatively analyzed. As a result, an improvement in AD in dogs was confirmed. The results of this study imply the possibility of commercialization of Synbio-glucan as a practical functional spray formulation for the treatment of AD in dogs that could be helpful in the effective management of the disease.

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Conflicts of Interest

The authors have no conflicting interests.

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