TANG [HUMANITAS MEDICINE]

Original Article

The regulatory effect of AST cream on atopic dermatitis-like skin disease.

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ABSTRACT

In this study, we investigated an inhibitory effect of AST cream on atopic dermatitis (AD) using a 2,4-dinitrochlorobenzene-induced AD murine model. Topical treatment with AST cream ameliorated the severity of AD-like lesional skin through decreases in infiltration of inflammatory cells and time of scratching behaviors. Also, AST cream reduced histamine and IgE levels in serum. The protein levels of IL-4 and IL-6 in AD-like lesional skin were suppressed by AST cream. These findings suggest that AST cream would be an alternative therapeutic agent for AD-like skin diseases.

Keywords AST cream; histamine; IgE; atopic dermatitis

1. INTRODUCTION

Atopic dermatitis (AD) as the most common recurrent inflammatory skin disease in the world (Lowa et al., 2018), aggravates quality of life and causes considerable strain such as psychological stress and high medical costs (Paton, 2017). Although many researchers have developed new oral drugs, patients with AD are steadily increasing. This may be due to the various causative factors and pathological pathways of AD. In addition, topical antibiotics commonly prescribed for AD are involved in fewer systemic side effects and lower risks of antibiotic resistance than oral antibiotics (Belloni Fortina and Neri, 2015; Venekamp et al., 2016). Thus, studies on topical application as an alternative therapy as well as oral administration have been also actively conducted (Choi and Kim, 2013; Ryu et al., 2018).

AST cream is mainly composed of processed-Cordyceps militaris and processed-Rumex crispus. Cordyceps militaris has reported to have anti-inflammatory and anti-cancer activities (Lee et al., 2015; Ng and Wang, 2005). Rumex crispus has reported to have anti-oxidant activities (Park et al., 2006). In addition, oral treatment with AST2017-01 which consists of processed-Cordyceps militaris and processed-Rumex crispus, has been reported to have beneficial effects on mice with AD and allergic rhinitis (Han et al., 2018; Kim et al., 2019).

We examined whether topical application of AST cream as a new treatment method would improve AD-like lesional skin in 2,4-dinitrofluorobenzene (DNFB)-induced murine model of AD.

2. MATERIAL AND METHODS

2.1. Animals

Animal study was approved from the animal care committee of Kyung Hee University [KHUASP (SE)-18-022]. DNFB (Sigma Chemical Co., St. Louis, MO, USA)-induced AD-like lesional skin were obtained from female BALB/c mice of 8 weeks (Dae-Han Experimental Animal Center). Acetone (100 μl) or 0.15% DNFB (100 μl) was topically applied to shaved abdominal skin. A week later, the shaved dorsal skin was applied with acetone (50 μl) or 0.15% DNFB (50 μl) twice a week for 3 weeks. At the same time, AST cream (20 mg/site, Gahwa Well Food Co., Chungbuk, Republic of Korea) was topically applied to the shaved dorsal skin three times a week for 3 weeks.

2.2. Histological analysis

The fixed lesional skins were embedded in paraffin. The slides were deparaffinized and rehydrated for staining with hematoxylin and eosin (H&E).

2.3. Histamine assay

Histamine levels in serum were measured according to o-phthaldialdehyde (Sigma Chemical Co.) spectrofluorometric procedure.

2.4. Cytokines assay

The levels of IgE (Pharmingen, Sandiego, CA, USA), TSLP (R & D system Inc., Minneapolis, MN, USA), IL-4 (Pharmingen), IL-6 (Pharmingen), and TNF-α (Pharmingen) in serum or lesional skin homogenates were measured with ELISA according to the manufacturer's instructions.

2.5. Caspase-1 activity assay

The caspase-1 activity in lesional skin was analyzed with a caspase-1 assay kit according to the manufacturer's instructions (R & D system Inc.).

2.6. Statistics

The results are expressed as the mean standard error of mean (SEM). Statistical analysis was performed by an independent t-test and ANOVA with Tukey post-hoc test using IBM SPSS v23 statistics software. Results with a p value <0.05 were considered significant.

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Received Jul 31, 2019; **Accepted** Aug 16, 2019; **Published** Aug 31, 2019
doi: http://dx.doi.org/10.5667/tang.2019.0015

3. RESULTS AND DISCUSSION

We investigated whether AST cream could improve AD-like symptoms. Figure 1 shows that topical application of DNFB induced AD-like symptoms including erosion. The topical application with AST cream ameliorated the symptoms in AD-like lesional skin. The topical application with AST cream decreased thickness of epidermis and infiltrations of inflammatory cells in lesional skin (Figure 2A). The time of scratching behavior was significantly reduced by the topical application with AST cream (Figure 2B, $p < 0.05$). Histamine and IgE induce scratching behaviors and aggravate the development of AD (Klein and Clark, 1999; Weber et al., 2005). Thus, we measured the histamine and IgE levels in serum. The topical application with AST cream significantly suppressed the serum histamine and IgE levels increased by DNFB (Figure 3, $p < 0.05$). However, serum TSLP, IL-4, IL-6, and TNF-α levels were not inhibited by the topical application with AST cream (Figure 4). AST cream did not decrease TSLP level in the lesional skin (Figure 5A). Interestingly, IL-4 and IL-6 levels were significantly suppressed by AST cream (Figure 5B and C, $p < 0.05$). Patients suffering from atopic dermatitis had higher levels of proinflammatory cytokines, such as IL-4 and IL-6 (Klonowska et al., 2018; Navarini et al., 2011). In addition, IL-4 and IL-6 were significantly associated with itching (Konda et al., 2015; Wong et al., 2017). Topical application of josamycin to AD lesions inhibited the development of AD-like skin lesions, suppressing IgE and IL-4 levels (Matsui et al., 2017). Thus, AST cream might regulate scratching behaviors by reducing IL-4 and IL-6 levels in the lesional skin. We finally examined a regulatory effect of AST cream on caspase-1 activity in the lesional skin. However, AST cream did not reduce the caspase-1 activity in the lesional skin (Figure 6).

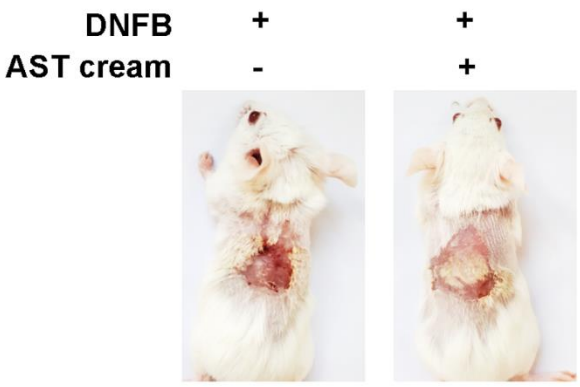

Fig. 1 Representative dorsal skin photographs

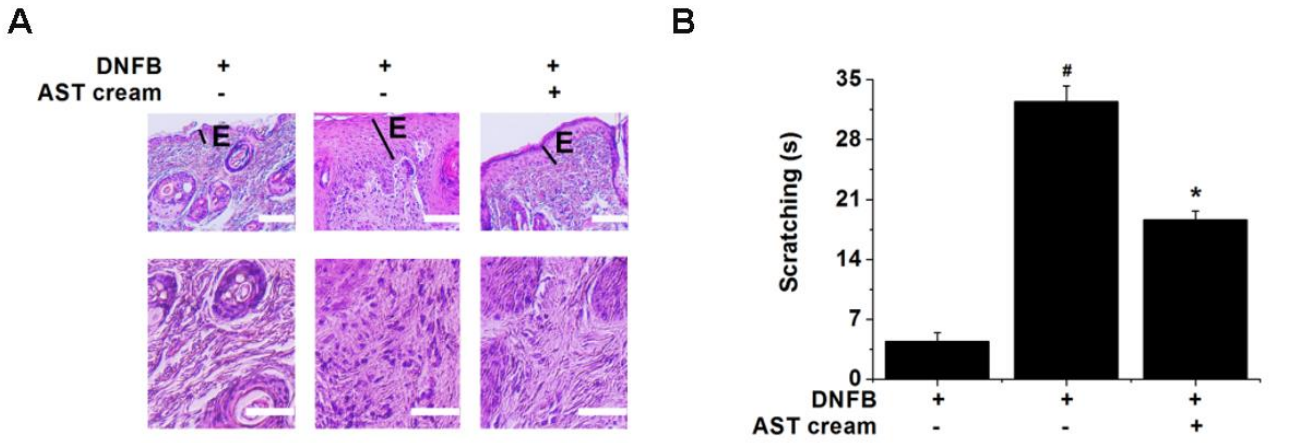

Fig. 2 (A) Histological features of lesional skin stained with H&E. E, epidermis. (B) The time of scratching behavior. $^{\#}p < 0.05$ vs vehicle group. $^{*}p < 0.05$ vs DNFB group.

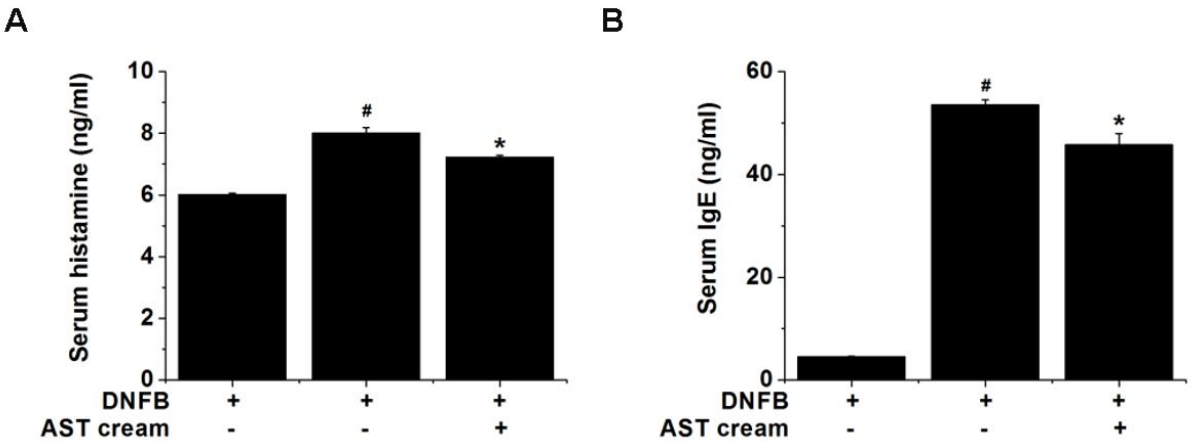

Fig. 3. (A) The serum histamine levels. (B) The serum IgE levels detected by ELISA. $^{\#}p < 0.05$ vs vehicle group. $^{*}p < 0.05$ vs DNFB group.

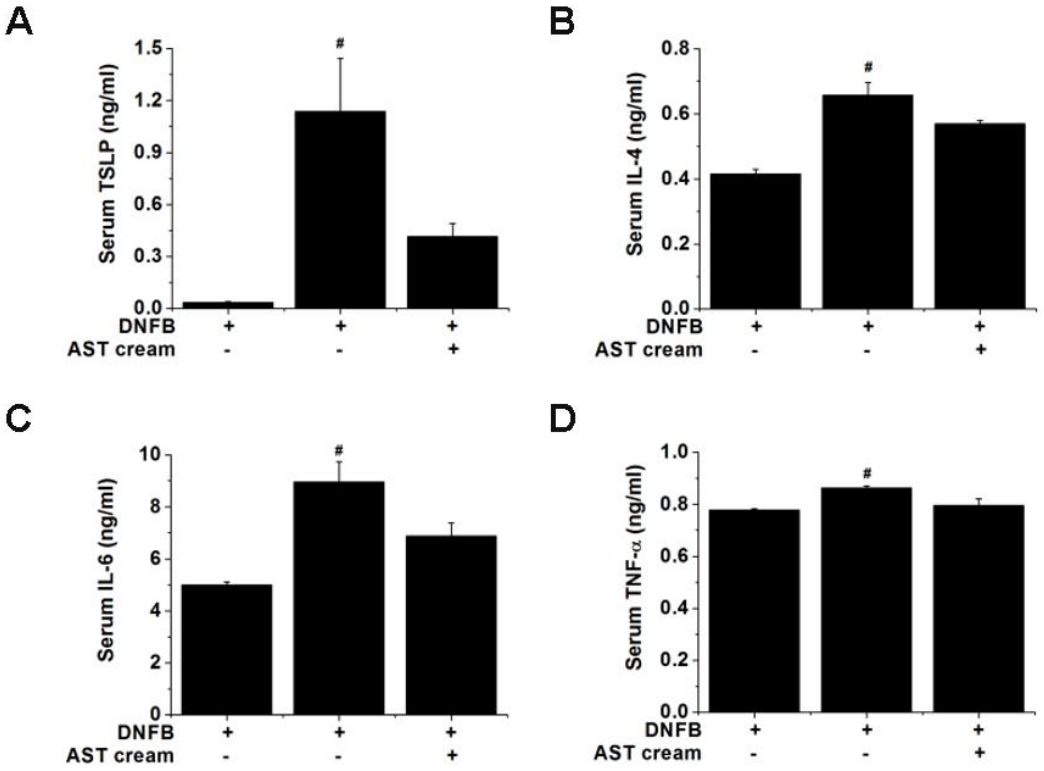

Fig. 4. The serum TSLP, IL-4, IL-6, and TNF-α levels detected by ELISA. #p < 0.05 vs vehicle group.

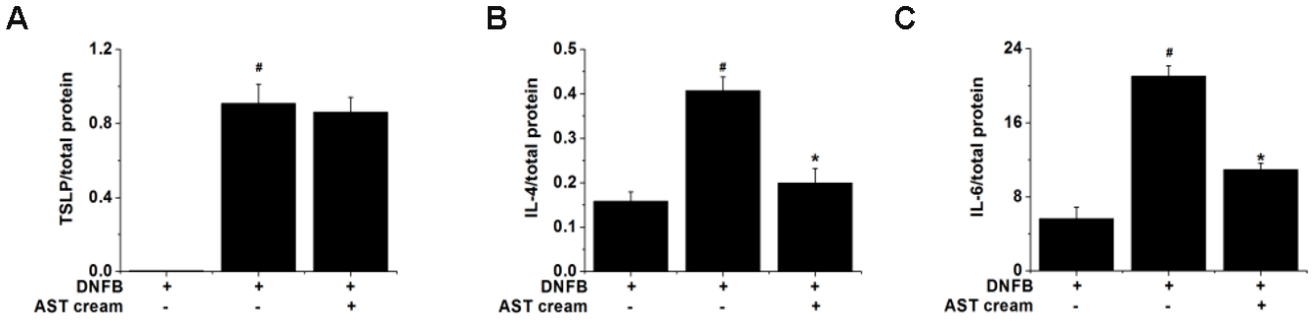

Fig. 5. The protein expression levels of TSLP, IL-4, and IL-6 detected by ELISA in the lesional skin. #p < 0.05 vs vehicle group. *p < 0.05 vs DNFB group.

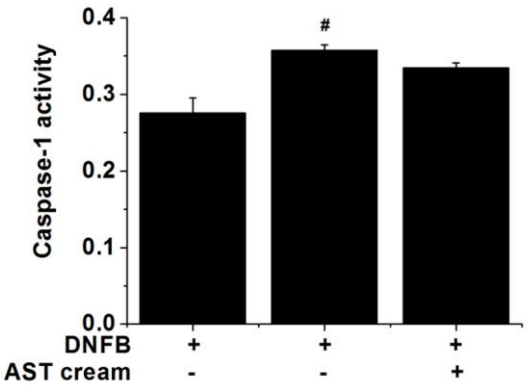

Fig. 6. Caspase-1 activity in the lesional skin. $^{\#}p < 0.05$ vs vehicle group.

4. CONCLUSION

We assume that AST cream improves AD-like symptoms through down-regulating histamine, IgE, IL-4, and IL-6 levels. Our findings suggest that the topical application with AST cream can serve as a potent therapeutic agent for AD-like skin diseases as an alternative therapy.

CONFLICTS OF INTEREST

The authors declared that they have no conflicts of interest to disclose.

FUNDING

This work was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) through High Value-added Food Technology Development Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) (116169-3).

REFERENCES

Belloni Fortina A, Neri L. Antibiotic therapy in the management of atopic dermatitis. G Ital Dermatol Venereol. 2015;150(3):321-5.

Choi JK, Kim SH. Rutin suppresses atopic dermatitis and allergic contact dermatitis. Exp Biol Med (Maywood). 2013;238(4):410-7.

Han NR, Moon PD, Yoo MS, Ryu KJ, Kim HM, Jeong HJ. Regulatory effects of chrysophanol, a bioactive compound of AST2017-01 in a mouse model of 2,4-dinitrofluorobenzene-induced atopic dermatitis. Int Immunopharmacol. 2018;62:220-6.

Kim HY, Jee H, Yeom JH, Jeong HJ, Kim HM. The ameliorative effect of AST2017-01 in an ovalbumin-induced allergic rhinitis animal model. Inflamm Res. 2019;68:387-95.

Klein P, Clark RAF. An Evidence-Based Review of the Efficacy of Antihistamines in Relieving Pruritus in atopic dermatitis. Arch Dermatol. 1999;135:1522-5.

Klonowska J, Gleń J, Nowicki RJ, Trzeciak M. New Cytokines in the Pathogenesis of Atopic Dermatitis-New Therapeutic Targets. Int J Mol Sci. 2018;19(10):3086.

Konda D, Chandrashekar L, Rajappa M, Kattimani S, Thappa DM, Ananthanarayanan PH. Serotonin and interleukin-6: Association with pruritus severity, sleep quality and depression severity in Prurigo Nodularis. Asian J Psychiatr. 2015;17:24-8.

Lee HH, Lee S, Lee K, Shin YS, Kang H, Cho H. Anti-cancer effect of Cordyceps militaris in human colorectal carcinoma RKO cells via cell cycle arrest and mitochondrial apoptosis. Daru. 2015;23:35.

Lowa A, Jevtic M, Gorreja F, Hedtrich S. Alternatives to animal testing in basic and preclinical research of atopic dermatitis. Exp Dermatol. 2018;27:476-83.

Matsui K, Tachioka K, Onodera K, Ikeda R. Topical Application of Josamycin Inhibits Development of Atopic Dermatitis-Like Skin Lesions in NC/Nga Mice. J Pharm Pharm Sci. 2017;20:38-47.

Navarini AA, French LE, Hofbauer GF. Interrupting IL-6-receptor signaling improves atopic dermatitis but associates with bacterial superinfection. J Allergy Clin Immunol. 2011;128(5):1128-30.

Ng TB, Wang HX. Pharmacological actions of Cordyceps, a prized folk medicine. J Pharm Pharmacol. 2005;57:1509-19.

Park DH, Jeong GT, Lee GM. Study of antimicrobial and antioxidant activities of Rumex crispus extract. Korean Chem Eng Res. 2006;44:81-6.

Paton DM. Dupilumab: human monoclonal antibody against IL-4Ra for moderate to severe atopic dermatitis. Drugs Today. 2017;53:477-87.

Ryu KJ, Yoou MS, Seo Y, Yoon KW, Kim HM, Jeong HJ. Therapeutic effects of Artemisia scoparia Waldst. et Kitaib in a murine model of atopic dermatitis. Clin Exp Dermatol. 2018;43(7):798-805.

Venekamp RP, Prasad V, Hay AD. Are topical antibiotics an alternative to oral antibiotics for children with acute otitis media and ear discharge? BMJ. 2016;352:i308.

Weber MB, Petry V, Weis L, Mazzotti NG, Cestari TF. Evaluating the relation between pruritus, serum IgE levels and severity of clinical manifestations in atopic dermatitis patients. An Bras Dermatol. 2005;80:245-8.

Wong LS, Wu T, Lee CH. Inflammatory and Noninflammatory Itch: Implications in Pathophysiology-Directed Treatments. Int J Mol Sci. 2017;18(7):1485.