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Original Article

Combined treatment of Taraxaci Herba and R7050 alleviates the symptoms of herpes simplex virus-induced Behçet's disease in rats

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ABSTRACT

Background: Behçet's disease (BD) is a chronic inflammatory systemic disease that affects multiple organs. The causes of BD are still unknown, but it is primarily characterized by autoimmune reaction in the blood vessels. Current research focuses on treatments that can reduce the non-typical inflammatory responses of BD. Nevertheless, studies on improving the inflammatory effect of BD using inflammation mechanisms are still insufficient. Therefore, we conducted the integrated treatments related to inflammation modulation and achieved alleviation of symptoms in BD mice.

Methods: To understand the complex etiology of BD and compare its management, the herpes simplex virus (HSV)-induced BD mouse model was used. In order to alleviate the inflammatory response in BD mice, Taraxaci Herba (TH, herbal medicine), R7050-a TNFα inhibitor, and a mixture of TH and R7050 were injected for 2 weeks repetitively. The SCORAD index was examined to evaluate the cutaneous inflammations. In addition, histological changes and inflammatory factors were analyzed.

Results: Repetitive injection of TH and/or R7050 reduced the symptoms of BD and significantly decreased IL-6, IL-1β, and TNFα in blood sera. Moreover, this treatment reduced the ulcers and the deterioration of skin.

Conclusions: The results of our study showed that the down-regulation of inflammatory factors is related to the control of immune responses in BD models, suggesting that a mixed drug treatment may be more effective in improving the condition of BD.

1. Introduction

Behçet's disease (BD) is a rare multigenetic disorder of unknown etiology. It is a multisystem inflammatory vasculitis characterized by recurrent oral and genital ulcerations, ocular lesions, and arthritis [1]. Although almost all organs might eventually be involved, the involvement of the central nervous system and eyes can worsen the prognosis; therefore, proper treatment is required [2]. A recent study indicated that several factors, including environmental pollution, infections, genetically determined phenotypes, and immune dysregulation, are related to the pathogenesis of BD [3].

The current treatment of BD is mainly based on the control of inflammation by using immunomodulatory or immunosuppressive agents. tumor necrosis factor alpha (TNFα), which is one of the multi-functional cytokines, promotes inflammatory response and mediates cell death by binding to TNF receptor 1, which triggers the extrinsic pathway of apoptosis [4,5]. Since the plasma level of TNFα is elevated in patients with inflammatory disease, TNFα has generated interest in the treatment of BD. However, anti-TNFα therapies are associated with challenges such as their availability, potential antigenicity, and tissue distribution [6].

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In traditional medicine, a comprehensive approach with herbal medicine has been used for the treatment of inflammatory diseases including allergy, asthma, multiple sclerosis, coeliac disease, glomerulonephritis, hepatitis, and inflammatory bowel disease [7,8]. Among them, Taraxaci herba (TH) is widely used as a traditional medicine for febrifuge and reducing edema [9]. TH is derived from the dried herba of Taraxacum mongolicum, and consists of taraxasterol, choline, inulin, and pectin [10]. It has been reported that TH can reduce heat and swelling and also remove toxic substances at the site of inflammation [11,12] Another prominent class of biologicals are the anti-TNF drugs that are applied in several inflammatory diseases characterized by dysregulated TNF levels. Among them, recent studies on R-7050 found that R7050 shows excellent effects as a TNFα antagonist. R-7050 was observed to be effective as an adjuvant therapy in the treatment of nerve damage as a TNF receptor complex inhibitor that improves the course after intracranial hemorrhage [13,14]. However, studies about the efficacy of TH and R7050 in improving the inflammatory effect in BD are still inadequate.

In patients with BD, inflammatory activation leads to rheumatoid disease, neurological deterioration, and mucosal skin symptoms, although the exact mechanism is unknown [15]. There are significant evidence-based data about the management of rheumatoid arthritis, ocular diseases, and mucocutaneous diseases in the animal models of BD. However, there is insufficient evidence regarding the treatment strategies for neurologic and vascular manifestations. Although therapies for reducing inflammation have been proposed, the quality of evidence is not high [16]. Emerging preclinical and clinical evidence on BD suggests a potential role of the anti-inflammatory response by the innate immune system to control the symptoms of BD; however, this idea remains largely unexplored.

The role of viral infections, such as herpes simplex virus (HSV) infection, in the pathogenesis of Behçet's disease (BD) has been investigated for many years. The clinical similarities between herpetiform ulcers in BD and ulcers due to HSV infection suggest an etiologic role of HSV in BD, and several studies have attempted to isolate HSV from the oral ulcers of patients with BD [2,3,17]. The species-specificity and inherent abnormality of human leukocyte antigen (HLA) molecules in mice model are also similar to the presence of genetic predisposition in BD patients [17].

The present study aimed to determine whether the direct use of TNFα inhibitor (R7050, a selective antagonist of TNFα/TNFR1 pathway), and TH could relieve symptoms of BD, and whether the levels of a related cytokine could be reduced by injecting the drug to further modulate the symptoms. The presence or absence of dermatitis in BD was scored using the SCORAD index, which includes erythema, edema/papulation, oozing/crusting, excoriation, lichenification, and dryness [18,19]. We also compared the results of integrated treatment using two drugs simultaneously.

2. Methods

2.1. Animals and induction of BD

All animal experiments were approved by the Institutional Animal Care and Use Committee of Yonsei University Health System (protocol number 2019–0093), and were performed in accordance with the guidelines for the ethical use of conscious animals in pain research published by the International Association for the Study of Pain. We used 4-week-old male ICR mice ($n = 40$, consisting of five groups of eight mice, Orient Bio, Seongnam, Gyeonggi-do, Korea) for this study. In each group, four mice per group were used for tissue staining, and the others were used for WB. Using the method described by Sohn et al. [20], we scratched the earlobes of the mice with a needle, and inoculated them with herpes simplex virus-2 (HSV-2) (KBPV-VR-84, Korea Bank for Pathogenic viruses, Seoul, Korea). Virus inoculation was performed four times within 2 weeks, which was followed by 4 weeks of observation. No HSV-inoculated mice were included in the control group. All mice were bred in temperature and light-controlled conventional BL3 rooms (20–22 °C; 12 h light cycle starting at 8:00 AM), and had free access to food and water. During the experimental period, the animals were closely observed and photographed.

2.2. Gross observation of BD symptoms

To classify the symptomatic mice as having BD, we used a previous classification [20]. Oral, genital, and other skin ulcers (including oozing and crusting ulcers) as well as ocular symptoms were classified as major indications. The presence of at least two major indications in a mouse were classified as BD. Symptomatic mice were photographed with a digital camera.

2.3. Drug (TNF receptor-1 inhibitor and Taraxaci herba) administration

The TNF Type-I receptor inhibitor, R7050 (Merck KGaA, Darmstadt, Germany), was dissolved in saline. The selected concentration of R7050 was referenced from previous studies [14,21]. In addition, anti-inflammatory herbal medicine, Taraxaci Herba (TH 1 kg, SQ-17,069–1–1, Green Pharm. Gyeonggi-do, Korea), was prepared, as described below. TH was boiled in distilled water (DW) up to three times the weight of the herb for 3 h and filtered using gauze. The filtered TH soup was lyophilized with a freeze dryer (FreeZone 6 plus Labconco, Kansas City, MO, US) [22]. Finally, 5 to 7% of TH was obtained in powder form. Saline, R-7050 (10 mg/kg), and TH (6 mg/kg) were administered repetitively via the intraperitoneal route for 2 weeks (four times). In addition, mice in the TH + *R*-7050 group were injected with each drug at 10-minute intervals. There was no HSV-inoculated healthy mouse in the control group.

2.4. Histology

After the mice were sacrificed, tissues were retrieved for post-fixation in 4% paraformaldehyde in 0.1 M phosphate buffer (4 °C), and then prepared in 30% sucrose in phosphate buffered saline over 24 h. Sagittal sections (12 μm) of the tissues were obtained using a cryostat (Microm HM525; Thermo Scientific, Waltham, MA, USA). Sections obtained at similar planes were used for haematoxylin and eosin staining. Tissue sections were washed in DW, followed by staining with haematoxylin to stain the nuclei and eosin to counterstain the cytoplasmic regions. Stained sections were examined using a microscope (Olympus BX40; Olympus, Tokyo, Japan). Then, eight tissue samples were randomly selected for each group, and the number of hair follicle cells in the 4X enlarged tissue pictures was counted and averaged.

2.5. Western blot analysis

Four weeks later, the mice were anesthetized with an intraperitoneal injection of a mixture of alfaxalone hydrochloride (40 mg/kg) and xylazine (10 mg/kg). To analyze the inflammatory factors in blood, samples were collected from the superior vena cava. One microliter of blood per mouse was collected and stored at −80 °C in polypropylene tubes until the western blot analysis. The microtubes were centrifuged for 5 min at 10,000 × *g*, and sera were collected. Samples were denatured in lithium dodecyl sulfate (LDS) buffer containing DTT, and loaded onto a NuPAGE® 4–12% Bis-Tris Mini gel to perform electrophoresis. Proteins were then transferred to polyvinylidene difluoridine (PVDF) membranes

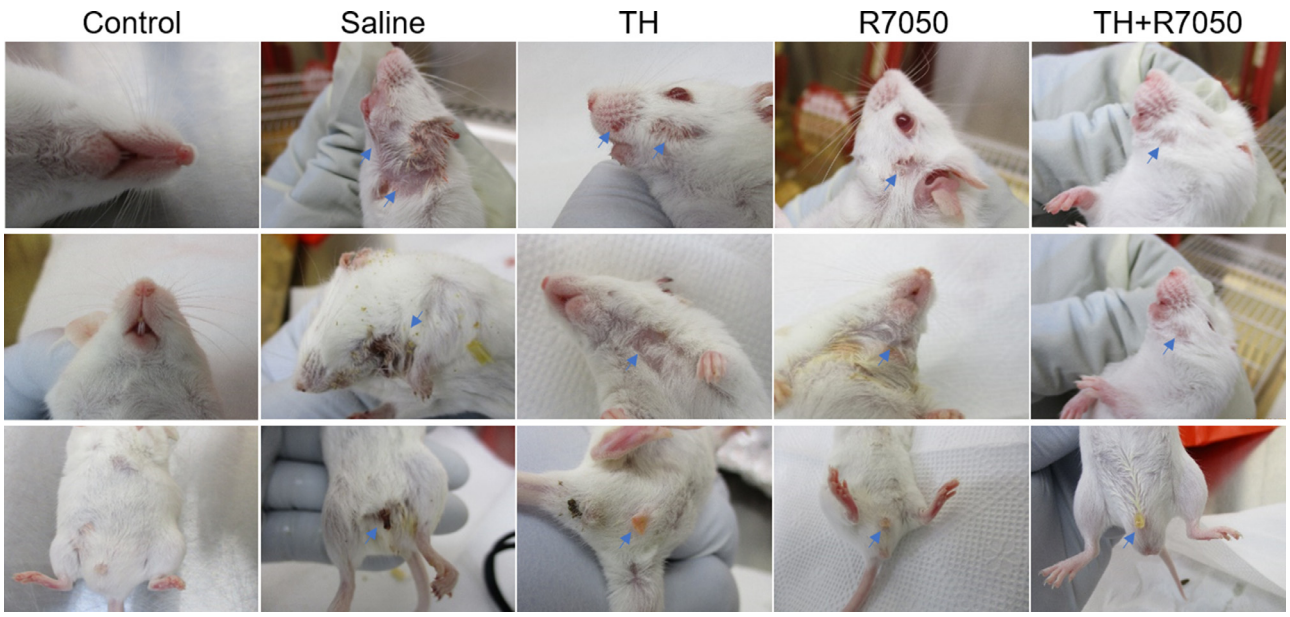

Fig. 1. Comparison of herpes simplex virus (HSV)-induced Behçet's disease (BD) symptoms in mice. In saline groups with BD symptoms, oral, skin, and genital skin ulcers and eye-related changes were observed. However, in the groups injected with TH or R7050 alone, BD symptoms were alleviated, and better outcomes were observed in the group injected with TH+R7050 together.

(0.45 μm, Merck KGaA, Darmstadt, Germany). The membranes were blocked with 5% BSA in TBST, and incubated overnight at 4 °C with a rabbit polyclonal primary antibody (IL-1β; abcam ab9722, IL-6; abcam 208,113, TNFα; abcam 66,579, Cambridge, UK). Next, the membranes were washed and incubated with an anti-rabbit IgG HRP-conjugated secondary antibody for 1 h at room temperature. Immuno-reactive proteins were revealed by enhanced chemiluminescence. The band recognized by the primary antibody was visualized by using LAS-4000 (Fuji Film Co, Ltd., Tokyo, Japan), and densitometry was measured with Multi Gauge software (Fuji Film Co, Ltd., Tokyo, Japan). To allow quantification across several gels, one sample was used as an internal calibrator, which was loaded on each gel and set to 100%.

2.6. Statistical analysis

Values are expressed as mean ± SEM, and were compared using one-way analysis of variance (ANOVA) followed by Dunnett's post hoc pairwise comparisons (Prism 8, GraphPad Software, San Diego, CA, US). A p value less than 0.05 was considered statistically significant.

3. Results

A multisystemic disorder with erythema, edema/papulation, oozing/crusting, excoriation, lichenification, and dryness appeared in HSV-inoculated mice. Mice with at least two major symptoms were classified as having BD-like symptoms [23].

3.1. Evaluation of multisystemic disorder in BD mice

To determine whether anti-inflammatory responses play a role in the development of HSV-induced BD symptoms, ICR mice were treated with a mixed solution of saline, TH, R7050, and TH+R7050 to suppress the inappropriate inflammatory responses. Symptoms developed in the inoculated mice, and these included oozing/crusting in the facial region, erythema on the scratched earlobe, ocular symptoms (e.g., hypopyon, iridoretinitis, and uveitis), as well as skin edema, lichenification, and papules on the earlobes, genitalia, and other regions, as reported [24]. (Fig. 1) For analysis, the SCORAD index (scoring for atopic dermatitis) method used in previous clinical studies [19] was applied for indexing the inflammatory symptoms of the skin. In the results as shown in Fig. 2, the scores in each group were summed up, and the contribution of each score was calculated. The index indicated erythema, edema/papulation, oozing/crusting, excoriation, lichenification, and dryness. It was observed that the symptoms were alleviated, and a highly significant difference was also shown between the saline group and the TH-, R7050-, and TH+R7050-injected mice (Control: 0 (0,0,0,0,0,0); Saline: 13.7 (2.2 ± 0.13, 2.1 ± 0.15, 3, 1.9 ± 0.11, 3, 1.5 ± 0.12); TH: 6.5 (1 ± 0.08, 1.5 ± 0.5, 0, 1.5 ± 0.2, 2.5 ± 0.31, 0); R7050: 5.2 (1.5 ± 0.2, 0, 1.5 ± 0.23, 1.2 ± 0.2, 1 ± 0.13, 0); TH+R7050: 3.5 (0, 1.5 ± 0.14, 2 ± 0.25, 0, 0, 0)). Following the injections of TH, R7050, and TH+R7050, skin ulcers gradually decreased and symptoms of alopecia improved in the TH and R7050 groups (Supplementay Fig. S1). The TH+R7050 group, which was injected with the two drugs simultaneously, also showed an improvement in lesions and inflammation.

3.2. Administration of anti-inflammatory drugs alleviates BD symptoms

We tried to describe the layers of skin tissue that appear in normal tissues and the morphological characteristics of individual layer. The tissue specimens show the stratum corneum and stratum lucidum, as well as the basement membrane and epidermal ridge, which make up the epithelial tissue (Fig. 3). Differences in skin lesions in mice with BD are presented in Fig. 3. Histopathological features of erythema, oozing, and crusting in BD are mainly characterized by changes due to the denaturation of the granular and stratum corneum. The results comparing the skin tissues in each group are shown in Fig. 3. In control rats, the appearance of normal skin tissue and the presence of hair follicles can be confirmed, and the distinction between the epidermis and dermis of the skin can be clearly distinguished. However, damaged skin tissues due to BD were observed in the saline group. Hair follicles from the dermis were blurred, and the structures of the epi-

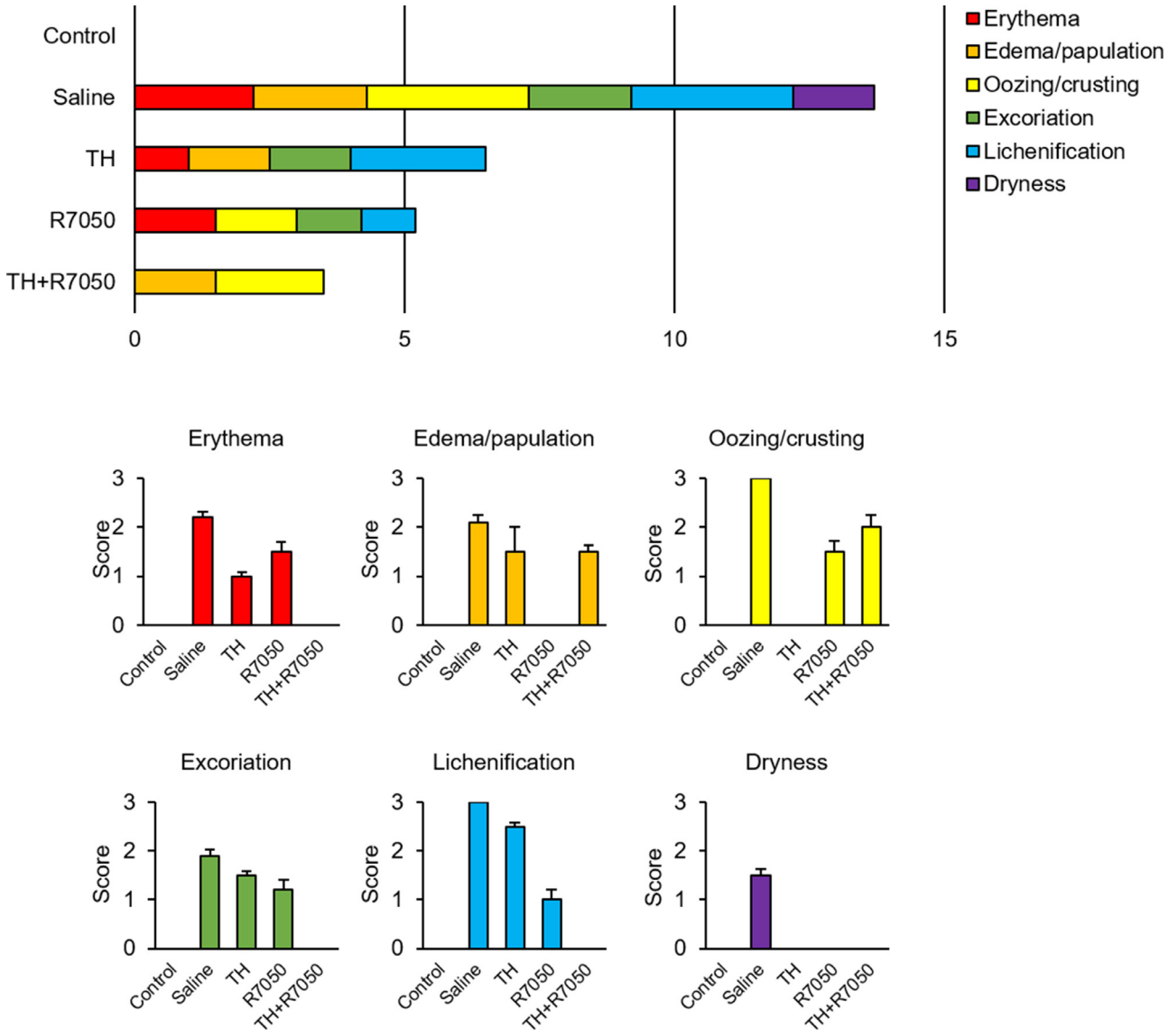

Fig. 2. SCORAD intensities of the experimental groups. Erythema, edema/papulation, oozing/crusting, excoriation, lichenification, and dryness were scored in each experimental group.

dermis and dermis were not clearly distinguished. In the TH and R7050 groups, hair follicles reappeared; however, it was difficult to observe changes in the structure of the dermis. In the skin tissue changes in the TH+R7050 group, the presence of distinct hair follicle cells was identified, and the dermal tissue layer was confirmed. An analysis was attempted by comparing the number of hair follicle cells in the tissues. Then, as shown in Fig. 4, eight tissue samples were randomly selected for each group, and the number of hair follicle cells in the 4X enlarged tissue pictures was counted and averaged (Control 43.25 ± 8.66; Saline 12.62 ± 2.82; TH 28.25 ± 1.48; R7050 29.5 ± 3.25; TH+R7050 34.62 ± 6.89). Following the symptoms of BD, the number of hair follicles was significantly reduced in the saline-treated group compared to the control group. In addition, the number of hair follicles increased in the TH and R7050 groups compared to the saline group. In the group in which TH and R7050 were administered together, a statistically significant increase in hair follicles was observed compared to that in the saline group; however, the difference was not significantly different from that of the groups that received TH or R7050 alone.

3.3. Down-regulated proinflammatory cytokines by drug administration in BD mice

To determine the levels of cytokines, sera were analyzed by western blot at 4 weeks after drug administration. The IL-1β level in the saline-injected group was significantly higher than that in the control and other groups (Control 1 ± 0.20; Saline 2.32 ± 0.40; TH 1.34 ± 0.30; R7050 1.33 ± 0.23; TH+R7050 1.05 ± 0.12). The level of IL-6 also increased significantly in the saline-injected group compared to other groups (Control 1 ± 0.13; Saline 2.18 ± 0.28; TH 1.15 ± 0.10; R7050 1.20 ± 0.07; TH+R7050 1.03 ± 0.15). In addition, the TNFα level in the saline injected group was also higher compared to other groups (Control 1 ± 0.27; Saline 2.33 ± 0.26; TH 1.16 ± 0.18; R7050 1.18 ± 0.20; TH+R7050 1.15 ± 0.32). The TH, R7050, and TH+R7050 groups showed significantly lower levels of proinflammatory cytokines compared to the saline-injected group. The BD model showed an overall increase in cytokines, but a significant decrease after injection of the drugs. Taken together, these results indicate that TH, R7050, and TH+R7050 may down-regulate proinflammatory cytokines in BD mice (Fig. 5).

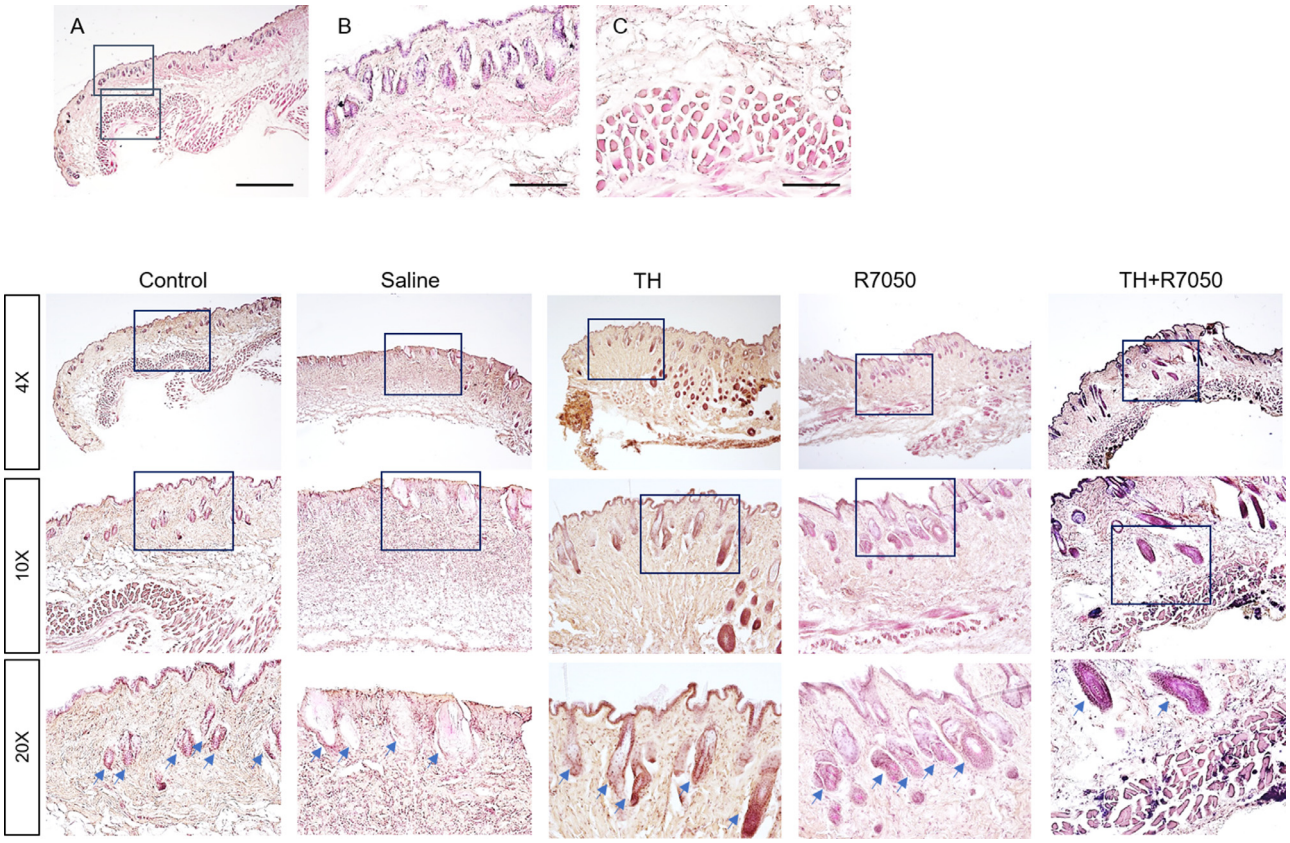

Fig. 3. Structural changes in the epidermis and dermis in the control group. A. Tissue observation after H&E staining with 4X microscope (scale bar: 1 mm). The two squares are enlarged and shown in B (upper) and C (bottom). B. Structure of the epidermis. Hair follicle cells could be easily observed in the epidermis of the skin (scale bar: 500 μm). C. Dermis and subcutaneous tissue of the skin. The composition of normal tissue can be observed (scale bar: 200 μm). Comparison of structural changes in the epidermis and dermis in each experimental group (Bottom 3 lines figures). Changes in the skin tissue in each group were observed and compared under the microscope (magnifications: 4 ×, 10 ×, and 20 ×). In the saline group, the structure of hair follicles appears to fade; however, in the TH, R7050, and TH+R7050 injection groups, clearer and increased number of hair follicles were observed.

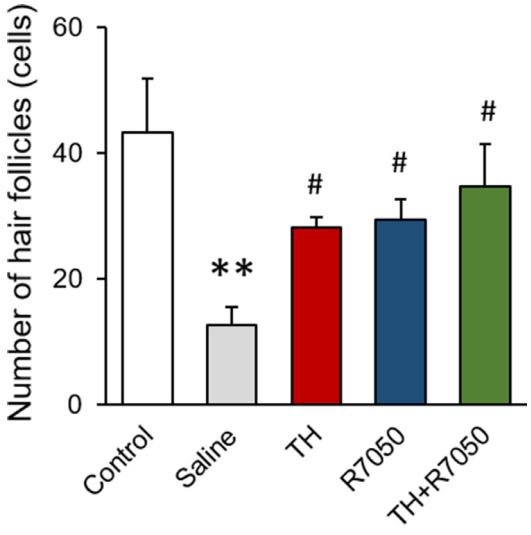

Fig. 4. Comparison of hair follicle cells between the experimental groups. The hair follicles were counted. Compared to the control group, the number of hair follicles was significantly higher in the BD model group injected with saline. In the experimental group administered TH, R7050, or TH+R7050 for 4 weeks, the number of hair follicles increased. Data are presented as means ± standard error of the mean (SEM). $^{**}p < 0.01$ vs. Control, $\#p < 0.05$ vs. Saline, as determined by one-way ANOVA followed by Dunnett's post hoc multiple comparison test to determine the significance.

4. Discussion

In our study, inflammatory responses induced by HSV and changes in skin tissue were observed in BD mice. By suppressing the abnormal inflammatory reactions caused by TNFα, an effective improvement in BD symptoms was observed. The effect of traditional medical treatment methods in reducing inflammation was observed using the SCORAD index method [19]. Administering anti-inflammatory drugs improved BD symptoms, such as genital and skin ulcers, and decreased the severity score. The changes in proinflammatory cytokines, which decreased after the drug administration, indicate that the symptoms of BD can be significantly alleviated by regulating the inflammatory responses.

Recent studies have suggested that proinflammatory cytokines might play a dominant role in triggering chronic autoimmune inflammation, and are considered essential for colitis and promotion of various inflammatory symptoms of the skin [25,26]. However, although biological treatment with anti-inflammatory agents has been shown to be effective in BD, research on the mechanisms of these beneficial responses being elucidated[27,28].. Among the cytokines being studied in BD, inhibition of the IL-1 family is the first area of interest. The IL-1 family contains 11 groups of cytokines that regulate many intracellular signaling pathways. IL-1α and IL-1β are type I receptors, and core receptor-accessory proteins are the most studied [27]. IL-1β is a major pre-inflammatory cytokine, and is known to up-regulate the innate immunity in response to expression of many chemokines and secondary mediators of inflammation, and to infectious agents [29]. IL-1β in BD is

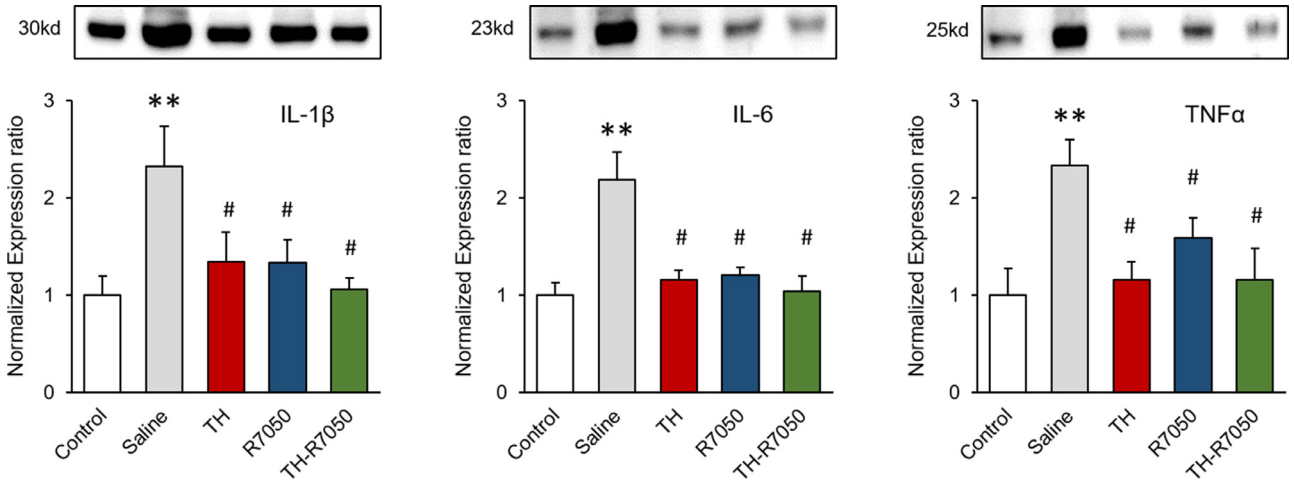

Fig. 5. Expression of inflammatory factors between the experimental groups. In comparing the changes of IL-1β, Il-6, and TNFα, a significant increase was observed in the BD groups injected with saline. However, the levels of IL-1β, Il-6, and TNFα were significantly decreased in the in the groups injected with TH or R7050 alone, and the TH+R7050-injected group. $^{**}p < 0.01$ vs. Control, $\#p < 0.05$ vs. Saline, as determined by one-way ANOVA followed by Dunnett's post hoc multiple comparison test to determine the significance.

considered to be mainly involved in BD due to the increased levels of IL-1β in the serum of BD patients and the fact that it suppresses IL-1β, leading to stable clinical remission [29]. TH is derived from the dried whole plant and consists of the taraxasterol, choline, inulin, and pectin. Its known effects include reduction of heat and swelling, as well as clearance of toxic materials at sites of inflammation. R-7050 is a cell-permeable TNF-α receptor antagonist that blocks TNF-α-induced binding of TNF-αRI with TNFαR-associated death domain protein and receptor interacting protein 1, blocking internalization of the TNFα-TNFαR complex. Previous studies have also indicated that these two drugs can pass easily through the BBB [14,30]. In our results, the TH+R7050 group showed a lower level of IL-1β compared to the group treated with TH or R7050 alone. These results suggest that independent application of TH and R7050 can reduce IL-1 β expression and inflammation; however, their combined use did not show significant effects. Among the available IL-1 blockers, the IL-1 receptor antagonists such as Anakira, canakinumab, and gevokizumab, which target the IL-1 molecule directly, have been used in BD patients; and they have shown successful IL-1 inhibitory effects, leading to an increased interest in anti-IL-1 agents for managing BD [31-34].

IL-6 consists of multiple cytokines secreted by various cell types, including T and B lymphocytes, macrophages, osteoblasts, fibroblasts, keratinocytes, and endothelial cells. It is involved in many immune pathways and plays a pivotal role in the regulation of various immune responses, aggravation of acute inflammation, and progression to relapse or chronic inflammatory responses [35,36]. Increased plasma IL-6 levels have been reported predominantly in BD patients, suggesting a correlation with disease activity in patients with predominantly neurological involvement [37]. Due to the effects of IL-6 on the immune system and inflammatory processes, IL-6 antagonism is considered a potential therapeutic strategy in various autoinflammatory and autoimmune disorders [38] [39]. In the present study, we focused on the changes in proinflammatory cytokine levels in BD using TH and R7050. The decrease in levels of IL-1β and IL-6 after TH and/or R7050 injections showed the immunomodulatory effects of TH and R7050, indicating that they might be effective for the alleviation of BD symptoms.

In particular, the inhibition of TNFα was reported to be successful in controlling inflammation in many patients [40]. The improved understanding of the mechanisms of TNFα pathway in BD has recently opened up new potential opportunities in terms of treatment. TNFα modulation therapy has been revolutionized by advances in the knowledge about the pathogenetic mechanisms of BD, namely, the dysfunction and over-secretion of a network of proinflammatory molecules, principally TNF-α [28]. Recently, anti-TNFα treatments, including infliximab and adalimumab, have been reported to be efficient in all severe and refractory BD manifestations [41]. Moreover, combination therapy of infliximab and methotrexate was administrated in Japan, and it showed short and long-term efficacy and tolerability, as assessed by abdominal computed tomography (CT) and colonoscopy. [42,43] Although many patients may benefit from treatment with anti-TNF drugs, a major challenge is the high number of patients who do not respond to therapy. Initial treatment responses have been reported to fail in 13 to 40% of patients, and it is difficult to observe the treatment effect in 50% of patients [44]. In this study, we used an anti-TNF agent and TH, which is a natural extract. Interestingly, in the group that received a mixture of TH and R7050, a decrease in the SCORAD score and recovery of skin tissue were observed on haematoxylin staining, with a marked improvement in the inflammatory cytokine changes. The hair follicle is a tunnel-shaped structure in the epidermis (outer layer) of the skin. Hair starts growing at the bottom of a hair follicle. The root of the hair consists of protein cells, and is nourished by the blood from nearby blood vessels. Skin abnormalities caused by inflammation induce redness and itchiness, and in severe cases, erythema, lichenification and hair loss. Our results showed the reduced inflammatory response-related follicle changes in the epidermal layer after treatment. These results suggest that the combination of a clinically used proinflammatory inhibitor and naturally-derived TH, as a treatment for BD, might be more effective in improving the symptoms of inflammation in BD. However, only a few animals were analyzed in this study, making it difficult to draw firm and definite conclusions. Therefore, further large controlled studies involving integrated treatment and longer follow-up periods are needed to corroborate these observations, and to confirm the efficacy and safety of these treatments, which provide a valuable knowledge for the treatment of BD.

In this study, using the HSV-induced BD model, we found that reducing the inflammatory response is a major factor in alleviating the symptoms of BD. In the current study, the potential of integrative medicine was expected by using effective anti-inflammatory drugs together. As a result, the reduction of inflammatory lesions of the skin could be detected by the regeneration of the hair folli-

cle, however the cytokine analysis did not show a significant synergic effect. Nevertheless, our experiment showed that it was not significant but effective in the recovery of skin edema and ulcers and changes in the inflammatory factors in BD animal model. Although, TH+R7050 could not show the significantly improve the inflammation-related molecular changes, our results indicated that the combination treatment of TH and R7050 could alleviates the symptoms of herpes simplex virus-induced Behçet's disease.

Author contributions

Conceptualization: MC, KKS and BHL. Methodology: MC, JHO and BHL. Formal investigation: MC and MP. Data analysis: MC and MP. Writing original draft: MC and MK. Writing review & editing: MC, BHL, and KKS.

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Conflict of interest

The authors have no conflict of interests.

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Ethical Statement

This study was approved by the Institutional Animal Care and Use Committee of Yonsei University Health System (protocol number 2019–0093).

Data Availability

The datasets used and/or analyzed in the current study are available from the corresponding author upon reasonable request.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.imr.2021.100720.

References

1. Greco A, De Virgilio A, Ralli M, et al. Behçet's disease: new insights into pathophysiology, clinical features and treatment options. *Autoimmun Rev*. 2018;17(6):567–575.
2. Sohn S, Lee ES, Bang D. Learning from HSV-infected mice as a model of Behcet's disease. *Clin Exp Rheumatol*. 2012;30:S96–103 3 Suppl 72.
3. Islam SMS, Sohn S. HSV-induced systemic inflammation as an animal model for Behcet's disease and therapeutic applications. *Viruses*. 2018(9):10.
4. Lorz C, Mehmet H. The role of death receptors in neural injury. *Front Biosci (Landmark Ed)*. 2009;14:583–595.
5. Cabal-Hierro L, Lazo PS. Signal transduction by tumor necrosis factor receptors. *Cell Signal*. 2012;24(6):1297–1305.
6. Lucas-Ruiz F, Galindo-Romero C, Salinas-Navarro M, González-Riquelme MJ, Vidal-Sanz M, Agudo Barriuso M. Systemic and intravitreal antagonism of the TNFR1 signaling pathway delays axotomy-induced retinal ganglion cell loss. *Front Neurosci*. 2019;13:1096 -1096.
7. Qiu J, Grine K. Complementary and alternative treatment for allergic conditions. *Prim Care*. 2016;43(3):519–526.
8. Amini F, Jaladat AM, Atarzadeh F, Mosavat SH, Parvizi MM, Zamani N. A review on the management of asthma in the Avicenna's canon of medicine. *J Complement Integr Med*. 2019(4):16.
9. Schütz K, Carle R, Schieber A. Taraxacum—A review on its phytochemical and pharmacological profile. *J Ethnopharmacol*. 2006;107(3):313–323.
10. Liu AP, Gou LX, Xue ZJ, Zheng YG, Guo L. [Quality evaluation of Taraxaci Herba based on fingerprint analysis and quantitative analysis of multi-components]. *Zhongguo Zhong Yao Za Zhi*. 2018;43(18):3715–3721.
11. Sohn S, Bang D, Lee SI, et al. Combined treatment with colchicine and Herba Taraxaci (Tarazacum mongolicum Hand.-Mazz.) attenuates Behcet's disease–like symptoms in mice and influences the expressions of cytokines. *Int. Immunopharmacol.*. 2003;3(5):713–721.
12. Hu C. Taraxacum: phytochemistry and health benefits. *Chinese Herbal Medicines*. 2018;10(4):353–361.
13. Gururaja TL, Yung S, Ding R, et al. A class of small molecules that inhibit TNFα-induced survival and death pathways via prevention of interactions between TNFαRI, TRADD, and RIP1. *Chem. Biol.*. 2007;14(10):1105–1118.
14. Jr. King MD, Alleyne CH, Dhandapani KM. TNF-alpha receptor antagonist, R-7050, improves neurological outcomes following intracerebral hemorrhage in mice. *Neurosci Lett*. 2013;542:92–96.
15. Davatchi F. Behçet's disease. *Int J Rheum Dis*. 2018;21(12):2057–2058.
16. Skef W, Hamilton MJ, Arayssi T. Gastrointestinal Behçet's disease: a review. *World J Gastroenterol*. 2015;21(13):3801–3812.
17. Kim DY, Cho S, Choi MJ, Sohn S, Lee ES, Bang D. Immunopathogenic role of herpes simplex virus in Behçet's disease. *Genet Res Int*. 2013 2013.
18. Kunz B, Oranje AP, Labrèze L, Stalder JF, Ring J, Taïeb A. Clinical validation and guidelines for the SCORAD index: consensus report of the European Task Force on Atopic Dermatitis. *Dermatology*. 1997;195(1):10–19.
19. Oranje AP, Glazenburg EJ, Wolkerstorfer A, de Waard-van der Spek FB. Practical issues on interpretation of scoring atopic dermatitis: the SCORAD index, objective SCORAD and the three-item severity score. *Br J Dermatol*. 2007;157(4):645–648.
20. Sohn S, Lee ES, Kwon HJ, Lee SI, Bang D, Lee S. Expression of Th2 cytokines decreases the development of and improves Behcet's disease-like symptoms induced by herpes simplex virus in mice. *J Infect Dis*. 2001;183(8):1180–1186.
21. EPoCitF Chain, Knutsen HK, Alexander J, et al. Risks to human and animal health related to the presence of deoxynivalenol and its acetylated and modified forms in food and feed. *EFSA J*. 2017;15(9):e04718.
22. Sohn S, Bang D, Lee SI, et al. Combined treatment with colchicine and Herba Taraxaci (Tarazacum mongolicum Hand.-Mazz.) attenuates Behcet's disease-like symptoms in mice and influences the expressions of cytokines. *Int Immunopharmacol*. 2003;3(5):713–721.
23. Oranje AP. Practical issues on interpretation of scoring atopic dermatitis: SCORAD Index, objective SCORAD, patient-oriented SCORAD and Three-Item Severity score. *Curr Probl Dermatol*. 2011;41:149–155.
24. Sohn S, Lee ES, Kwon HJ, Lee SI, Bang D, Lee S. Expression of Th2 cytokines decreases the development of and improves Behçet's disease-like symptoms induced by herpes simplex virus in mice. *J Infect Dis*. 2001;183(8):1180–1186.
25. Cua DJ, Sherlock J, Chen Y, et al. Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. *Nature*. 2003;421(6924):744–748.
26. Yen D, Cheung J, Scheerens H, et al. IL-23 is essential for T cell–mediated colitis and promotes inflammation via IL-17 and IL-6. *J Clin Invest*. 2006;116(5):1310–1316.
27. Caso F, Costa L, Rigante D, et al. Biological treatments in Behçet's disease: beyond anti-TNF therapy. *Mediators Inflamm 2014*. 2014 -107421.
28. Pineton de Chambrun M, Wechsler B, Geri G, Cacoub P, Saadoun D. New insights into the pathogenesis of Behçet's disease. *Autoimmun Rev*. 2012;11(10):687–698.
29. Dinarello CA, van der Meer JW. Treating inflammation by blocking interleukin-1 in humans. *Semin Immunol*. 2013;25(6):469–484.
30. Zhang X, Hong YL, Xu DS, et al. A review of experimental research on herbal compounds in amyotrophic lateral sclerosis. *Phytother Res*. 2014;28(1):9–21.
31. Moll M, Kuemmerle-Deschner JB. Inflammasome and cytokine blocking strategies in autoinflammatory disorders. *Clin Immunol*. 2013;147(3):242–275.
32. Alibaz-Oner F, Sawalha AH, Direskeneli H. Management of Behçet's disease. *Curr Opin Rheumatol*. 2018;30(3):238–242.
33. Park Y, Cheon JH. Update on the treatment of Behcet's disease of the small bowel with biologic agents. *Curr Gastroenterol Rep*. 2020;22(5):24.
34. Tugal-Tutkun I, Pavesio C, De Cordoue A, Bernard-Poenaru O, Gül A. Use of Gevokizumab in patients with Behçet's disease uveitis: an international, randomized, double-masked, placebo-controlled study and open-label extension study. *Ocul Immunol Inflamm*. 2018;26(7):1023–1033.
35. Atienza-Mateo B, Calvo-Río V, Beltrán E, et al. Anti-interleukin 6 receptor tocilizumab in refractory uveitis associated with Behçet's disease: multicentre retrospective study. *Rheumatology (Oxford)*. 2018;57:856–864 5.
36. Tanaka T, Narazaki M, Kishimoto T. Interleukin (IL-6) Immunotherapy. *Cold Spring Harb Perspect Biol*. 2018(8):10.
37. Talaat RM, Ashour ME, Bassyouni IH, Raouf AA. Polymorphisms of interleukin 6 and interleukin 10 in Egyptian people with Behcet's disease. *Immunobiology*. 2014;219(8):573–582.
38. Woodrick RS, Ruderman EM. IL-6 inhibition for the treatment of rheumatoid arthritis and other conditions. *Bull NYU Hosp Jt Dis*. 2012;70(3):195–199.

39. Palizgir MT, Akhtari M, Mahmoudi M, Mostafaei S, Rezaiemanesh A, Shahram F. Curcumin reduces the expression of interleukin 1β and the production of interleukin 6 and tumor necrosis factor alpha by M1 macrophages from patients with Behcet's disease. *Immunopharmacol Immunotoxicol*. 2018;40(4):297–302.
40. Hatemi G, Silman A, Bang D, et al. Management of Behçet disease: a systematic literature review for the European League Against Rheumatism evidence-based recommendations for the management of Behçet disease. *Ann Rheum Dis*. 2009;68(10):1528–1534.
41. Vallet H, Riviere S, Sanna A, et al. Efficacy of anti-TNF alpha in severe and/or refractory Behçet's disease: multicenter study of 124 patients. *J Autoimmun*. 2015;62:67–74.
42. Naniwa T, Iwagaitsu S, Kajiura M. Long-term efficacy and safety of add-on tacrolimus for persistent, active rheumatoid arthritis despite treatment with methotrexate and tumor necrosis factor inhibitors. *Int J Rheum Dis*. 2018;21(3):673–687.
43. Iwata S, Saito K, Yamaoka K, et al. Efficacy of combination therapy of anti-TNF-α antibody infliximab and methotrexate in refractory entero-Behçet's disease. *Mod Rheumatol*. 2011;21(2):184–191.
44. Ding NS, Hart A, De Cruz P. Systematic review: predicting and optimising response to anti-TNF therapy in Crohn's disease - algorithm for practical management. *Aliment Pharmacol Ther*. 2016;43(1):30–51.