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The Traditional Herbal Medicine Saireito Exerts Its Inhibitory Effect on Murine Oxazolone-Induced Colitis via the Induction of Th1-Polarized Immune Responses in the Mucosal Immune System of the Colon

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Key Words
Oxazolone-induced colitis · Saireito · SOCS-3 · Th1/Th2 balance · Ulcerative colitis

Abstract
Background: Ulcerative colitis is an intractable inflammatory colonic disease, and its etiology remains unclear. Saireito, a traditional herbal medicine, is widely used for treating ulcerative colitis in Japan. We analyzed the immunological characteristics of an oxazolone (OXZ)-induced colitis (OC) model and examined the effects of sareito on this model. ***Methods:*** OXZ was injected into the colon of BALB/c mice. Saireito was orally administered once a day for 3 consecutive days. Colitis was assessed by scoring the symptoms and macroscopic findings. The transcription patterns in the middle colon and spleen were analyzed with global transcriptome analysis and real-time polymerase chain reaction (PCR). ***Results:*** The above-mentioned scores were increased in the OC mice. The transcription levels of Th2 cytokines were significantly upregulated in the spleen and middle colon of the OC mice, whereas those of the Th1 cytokine interferon (IFN)-γ decreased in the spleen and increased in the middle colon. Saireito significantly ameliorated OC. In the middle colon of the saireito-treated mice, enhanced expression of Th2 cytokine mRNAs was markedly downregulated, while that of IFN-γ mRNA was further upregulated. In contrast, in the spleen, saireito had no effect on the transcription of either type of cytokine. After global transcriptome analysis, real-time PCR analysis revealed that saireito greatly downregulated the enhanced expression of the suppressor of cytokine signaling (SOCS)-3 mRNA in the middle colon of OC mice. ***Conclusions:*** Saireito exhibits inhibitory effects on OC by the induction of Th1-polarized immune responses in the mucosal immune system of the colon.

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Introduction

Ulcerative colitis (UC) is a continuous idiopathic inflammatory bowel disease (IBD) that is restricted to the colon. Although the etiology of UC still remains largely unknown, it has been suggested that a combination of genetic susceptibility factors and the activation of the mucosal immune system in response to intraluminal antigens of bacterial and dietary origin along with persistent pathologic cytokine production contributes to the initiation and chronification of UC [1–3].

Currently, patients with UC are usually treated with 5-aminosalicylic acid and/or a corticosteroid. Corticosteroids are more effective than 5-aminosalicylic acid in inducing clinical and endoscopic remission in the treatment of UC. However, the prolonged use of corticosteroids may be limited by potential adverse effects. Corticosteroid therapy influences many metabolic processes. The precise mechanisms underlying the effect of corticosteroids are not yet known, but serious side effects secondary to their prolonged use may occur in various situations. The literature shows a general agreement with the higher incidence of osteoporosis, fracture [4], and cataract [5]. Some aspects of these diseases deserve special consideration in younger patients; the side effects of corticosteroids may be more severe after prolonged medication in younger patients than in adults [6].

Various alternative pharmacological approaches have been put forward to decrease the dosage of steroids in order to decrease their side effects and, more importantly, to avoid colectomy. In the past decade, IBD therapy has significantly progressed, and new treatments, such as immunosuppressive therapy, have been successfully introduced [7].

There are many patients with UC who are interested in complementary and alternative medicine because they are dissatisfied by the current conventional therapies [8]. In Japan, traditional Japanese herbal medicine, namely, Kampo medicine, is widely used in the treatment of UC, and many studies have reported that Kampo medicine improves UC [9]. In particular, saireito, which is widely used for the treatment of inflammatory diseases such as rheumatoid arthritis [10], systemic lupus erythematodes [10], and nephrotic syndrome [11], has already been reported to improve the symptoms associated with UC, e.g. diarrhea and hematochezia, and improve the endoscopic findings and quality of life, and reduce the dosage of corticosteroids administered in combination therapy [12, 13]. However, the precise mechanisms underlying the therapeutic effects of saireito remain largely unclear.

The pathogenesis of human IBD as well as that of Crohn's disease (CD) and UC has not been fully elucidated [14]. In CD, the responding T cells exhibit a type 1 T-helper cell (Th1) phenotype and hence produce large amounts of interferon-γ (IFN-γ); however, in UC, the responding T cells are not as well defined, and the cytokine profiles remain to be determined. However, the pathogenesis of UC is considered to be mediated primarily by type 2 T-helper cell (Th2) immune responses even if Th1 immune responses are involved in UC pathogenesis. This is because some murine colitis models clearly resemble human UC at the histopathological level in terms of the production of excess Th2 cytokines, whereas Th1-mediated colitis models have no similarity to human UC [15, 16].

Experimental animal models are indispensable for the accurate understanding of the pathogenesis of human diseases. In order to understand the etiology of UC, there should be an increased focus on experiments with animal models since such an approach facilitates easy understanding of the pathophysiology of UC and the elucidation of the pharmacological mechanisms underlying UC [17]. The experimental models of IBD are mainly known as the genetically modified and chemically induced models. An oxazolone (OXZ)-induced colitis model, which is one of the chemically induced colitis models, is considered, in some aspects, similar to human UC on the basis of the histological features of inflamed tissue and an elevated production of Th2 cytokines by lamina propria T cells [17, 18].

In this study, we examined the anti-inflammatory effects of saireito on an OXZ-induced colitis model of BALB/c mice.

Materials and Methods

Animals

Male BALB/c mice (5 weeks old) were purchased from Japan SLC (Shizuoka, Japan). All mice were housed in the experimental animal facility at the University of Toyama. All experimental procedures were performed in accordance with the standards established by the Guide for the Care and Use of Laboratory Animals of the University of Toyama.

Agents

4-Ethoxymethylene-2-phenyl-2-oxazolin-5-one (OXZ) was purchased from Sigma-Aldrich (St. Louis, Mo., USA). Ethanol and sodium methylcellulose were purchased from Wako Pure Chemical Industries (Osaka, Japan). Saireito is a prescription drug covered under the National Health Insurance Plan in Japan and was provided by Tsumura (Tokyo, Japan). According to the

Table 1. Assessment of colitis

a Criteria for disease activity score (DAS)

Score	Weight loss	Fecal consistency	Rectal bleeding
0	none	normal	normal
1	1–5%		
2	5–10%	loose feces	
3	10–20%	mild diarrhea	mild bleeding
4	>20%	severe diarrhea	severe bleeding
12	death		

b Criteria for macroscopic scoring of colonic damage (CDS)

Score	Colitis
0	no damage
1	hyperemia or slight bleeding and thickening of the bowel wall
2	bleeding or erosion
3	one site of ulceration or severe erosion
4	severe ulceration or tissue necrosis
8	death

Score	Adhesions	Mega-intestine
0	no damage	no damage
1	mild	narrowness
2	involving several bowel loops	mega-intestine

manufacturer's information, saireito is a water extract of 6.0 g of herbal powder; the extract is prepared from a decoction of 12 mixed medicinal herbs listed in the following: *Bupleuri radix*, 7.0 g; *Pinelliae tuber*, 5.0 g; *Alismatis rhizoma*, 5.0 g; *Scutellariae radix*, 3.0 g; *Ginseng radix*, 3.0 g; *Zizyphi fructus*, 3.0 g; *Poria*, 3.0 g; *Polyporus*, 3.0 g; *Atractylodis lanceae rhizoma*, 3.0 g; *Cinnamomi cortex*, 2.0 g; *Glycyrrhizae radix*, 2.0 g, and *Zingiberis rhizoma*, 1.0 g.

Induction of Colitis and Drug Administration

OXZ-induced colitis (OC) was induced in BALB/c mice using a modification of a previously described method [15, 19, 20]. A low dosage of OXZ was employed to reduce the high mortality in the induction of colitis encountered with high-dose OXZ. To be concrete, in order to presensitize the mice, the abdominal skin of the mice was shaved, and 50 μl of a 3% (w/v) solution of OXZ in 100% ethanol was applied on the shaved area (day 0). After 7 days of presensitization (day 7), 1-day-fasted mice were rechallenged with an intrarectal injection of 100 μl of 0.5% OXZ dissolved in 0.9% NaCl and mixed with an equal volume of ethanol (50% ethanol) while they were under light anesthesia with diethyl ether. A polyurethane umbilical catheter (Natsume, Tokyo, Japan) was used for the intrarectal administration, and it was inserted 4 cm into the lumen of the colon via the anus. Saireito [suspended in 0.5% (w/v) methylcellulose solution] was orally administered to the mice once a day. It was given 1 h before the instillation of OXZ enema on day 7 and on 3 subsequent days (days 7–9). The mice were sacrificed on day 10, and their tissues excised.

Assessment of Colitis

Clinical assessment of OC in the mice was according to the criteria for the disease activity score (DAS). The parameters assessed were change in body weight, stool consistency/diarrhea, and blood in feces (table 1a). At necropsy, colonic damage was assessed according to the criteria for the macroscopic scoring of colonic damage (CDS, table 1b), based on the degree of inflammation and the presence of edema and/or ulcerations.

Expression of Cytokine mRNA

The middle colon and spleen were washed with ice-cold saline, frozen in liquid nitrogen, and stored at –80°C. Total RNA was extracted from these tissues using Sepasol Super (Nacalai, Tokyo, Japan) according to the manufacturer's instructions. Reverse transcription was performed using the Exscript RT reagent kit (Takara, Tokyo, Japan) and random primers; real-time polymerase chain reaction (PCR) was subsequently performed. Real-time PCR amplification of interleukin (IL)-4, IL-5, IL-10, IFN-γ, suppressor of cytokine signaling (SOCS)-3, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was performed using SYBR Premix Ex Taq (Takara). The following primer pairs were used: IL-4, forward: 5′-GGTCTCAACCCCCAGCTAGT-3′, and reverse: 5′-CGCCGATGATCTCTCTCAAGTGAT-3′; IL-5, forward: 5′-GAAGTGTGGCGAGGAGAGAC-3′, and reverse: 5′-GCACAGTTTTGTGGGGTTTT-3′; IL-10, forward: 5′-GGCCCTTTGCTATGGTGTCC-3′, and reverse: 5′-AAGCGGCTGGGGGATGAC-3′; IFN-γ, forward: 5′-CGGCACAGTCATTGAAAGCCTA-3′, and reverse: 5′-GTTGCTGATGGCCTGATTGTC-3′; SOCS-3, forward: 5′-AGCTAATGAAACCTCGCAGATCC-3′, and reverse: 5′-AGCTCACCAGCCTCATCTGTCTC-3′, and GAPDH, forward: 5′-AAATGGTGAAGGTCGGTGTG-3′, and reverse: 5′-TGAAGGGGTCGTTGATGG-3′. Real-time PCR was carried out using Mx3000p (Stratagene, La Jolla, Calif., USA). The PCR reaction conditions were 10 s at 95°C, followed by 40 cycles at 95°C for 5 s and at 60–63°C for 20 s. For each sample, the target mRNA was normalized to the GAPDH mRNA, which was the internal control. Results were expressed as relative ratio to the normal group average.

Global Transcriptome Analysis

Total RNA was extracted from the middle colon as described above. The mRNAs extracted from 4 mice of each group were mixed. Microarray analysis was performed using GeneChip Mouse Genome 430 2.0 Array (Affymetrix, Santa Clara, Calif., USA) [21]. Data were analyzed using High-Resolution Scanning GeneChip Operating Software (version 1.3).

Statistical Analyses

Data are expressed as means ± SE. Either Student's unpaired t test as parametric test, Mann-Whitney's test as nonparametric test or one-way ANOVA followed by Dunnett's t test for multiple comparisons was used to determine statistical significance ($p < 0.05$) between means. n indicates the number of mice.

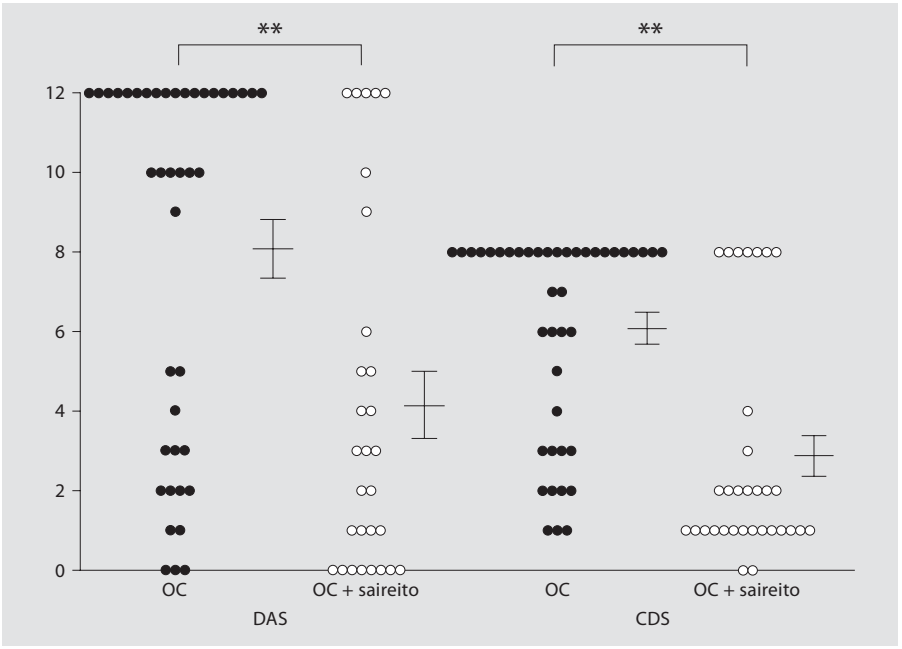

Fig. 1. Effect of oral treatment with saireito on the DAS and CDS in OC (for further details, see table 1). OC mice showed significantly increased DAS and CDS. Saireito significantly reduced the increased DAS and CDS in OC mice (OC + saireito). Means ± SE (DAS: OC: n = 41; OC + saireito: n = 29; CDS: OC: n = 42; OC + saireito: n = 32, ** $p < 0.01$).

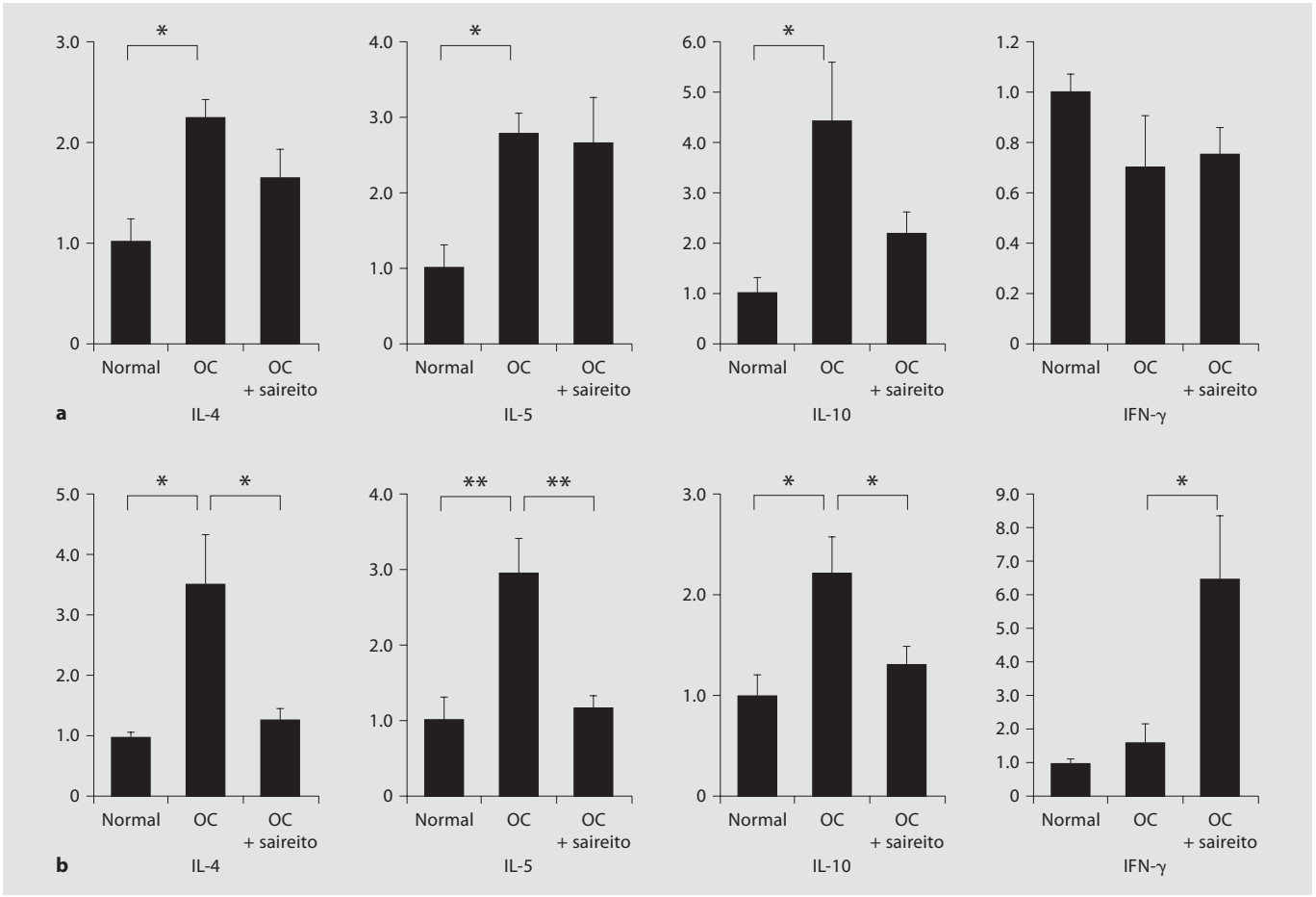

Fig. 2. Expression of cytokine mRNA in the spleen (**a**) and middle colon (**b**) in OC mice with/without saireito treatment. Means ± SEM (normal: n = 3, OC: n = 4, OC + saireito: n = 4; * $p < 0.05$, ** $p < 0.01$).

Results

Saireito Treatment Ameliorates OC

BALB/c mice intrarectally exposed to OXZ exhibited rapid onset of weight loss, loose feces/diarrhea, and hematochezia. The OXZ-treated mice rapidly developed colitis involving only the distal 50% of the colon; this disease was characterized by reddening, erosion, and superficial ulceration of the colon. DAS, the clinical colitis score, was significantly higher in the OXZ enema-treated mice than in the vehicle (50% ethanol) enema-treated mice (4.6 ± 1.8, $n = 11$, vs. 9.3 ± 1.3, $n = 21$; $p < 0.05$). Similarly, the CDS score was significantly higher for OXZ enema-treated mice than for vehicle enema-treated mice (3.3 ± 1.2, $n = 11$, vs. 7.2 ± 0.9, $n = 21$; $p < 0.01$). Oral treatment with saireito resulted in significant abrogation of DAS (8.0 ± 0.7, $n = 41$, vs. 4.1 ± 0.8, $n = 29$; $p < 0.01$; fig. 1). Furthermore, orally administered saireito significantly decreased the histological colitis score CDS (6.0 ± 0.4, $n = 42$, vs. 2.8 ± 0.5, $n = 32$; $p < 0.01$; fig. 1).

Transcription of Cytokines in the Spleen and Colon of OC Mice

To examine the change in immunological profiles in the OC mice, cytokine mRNA expression was measured by real-time PCR. The spleen was considered as the systemic immune system, and the middle colon adjacent to the site most markedly affected with colitis as the mucosal immune system. In the spleen of the colitis mice, mRNA levels of IL-4, IL-5, and IL-10 (Th2 cytokines) were significantly upregulated (fig. 2a), whereas that of IFN-γ (Th1 cytokine) was decreased (fig. 2a). On the other hand, in the middle colon of the OC mice, the levels of Th2 cytokine mRNAs were significantly upregulated, while the Th1 cytokine mRNAs were slightly upregulated (fig. 2b); these findings indicate that the Th1/Th2 balance was shifted toward Th2 dominance in the systemic and mucosal immune systems of the OC mice.

Effect of Saireito on the Cytokine Transcription in the Spleen and Colon of OC Mice

We evaluated the effect of orally administered saireito on the cytokine profiles in the OC mice. Saireito did not significantly affect the transcription levels of Th1 and Th2 cytokines in the spleen of the OC mice (fig. 2a). On the other hand, in the middle colon of the OC mice, saireito markedly decreased the expression of Th2 cytokine mRNAs, while it further greatly upregulated the expression of IFN-γ mRNA (fig. 2a).

To further investigate the pharmacological mechanism underlying the therapeutic effect of saireito on OC, we examined the global mRNA expression patterns in the middle colon of the non-treated, OC, and saireito-treated OC mice using the Affymetrix GeneChip Mouse Genome 430 2.0 Array. The expression levels of 12 genes were elevated >3-fold in the OC mice and had decreased in the saireito-treated OC mice (table 2a). In contrast, 35 genes were downregulated by less than one third in the OC mice and upregulated in the saireito-treated OC mice (table 2b). Most of these genes are associated with cell cycles or their roles have not yet been elucidated. Interestingly, the expression of the SOCS-3 gene, which plays an important role in regulating the onset and maintenance of Th2-related immune diseases, was influenced by the OXZ and saireito treatments.

Effect of Saireito on SOCS-3 mRNA Expression in the Colon of OC Mice

To explain the effect of saireito on SOCS-3 mRNA expression in the middle colon, real-time PCR was performed for a more detailed quantitative analysis of transcription. A significant increase in the SOCS-3 mRNA expression was observed in the middle colon of the OC mice, and orally administered saireito considerably reduced upregulated SOCS-3 mRNA expression (fig. 3).

Discussion

Our results conclusively demonstrate that saireito, which is a traditional Japanese herbal medicine, is an effective therapy for OC, and the mechanism by which it exerts its inhibitory effect involves, at least in part, the novel mechanism of suppressing SOCS-3 expression in the colon. These findings support the clinical effects of saireito on human UC because in Japan, saireito is actually used for the treatment of human UC.

With regard to the mucosal immune system in OC models, there are several reports on the cytokine profiles in the colon. In the SJL/J mouse OC model, the production of Th2 cytokines IL-4, IL-5, and IL-13 but not that of the Th1 cytokine IFN-γ was elevated in the unstimulated and anti-CD3/anti-CD28-stimulated lamina propria T cells of the inflamed colon [15]. In the C57BL/10 mouse OC model, the lamina propria mononuclear cells of the colon produce large amounts of IL-4, IL-5, and IL-13 but not IFN-γ by the stimulation with the anti-CD3/anti-CD28 antibodies [20]. Furthermore, in the inflamed colon of the C57BL/6 mouse OC model, mRNA expression

Table 2. Exhaustive examination of gene expression after saireito administration in OC

a Upregulation of gene expressions (>3-fold) by OXZ induction and downregulation after saireito administration

Accession	Gene definition
AV002218	arginase type II
BB349272	CLIP-associating protein 2
N M_007705	cold-inducible RNA-binding protein
BB543028	E2F transcription factor 2
N M_033648	FXYD domain-containing ion transport regulator 4
BB485297	IFN-γ-induced GTPase
N M_019440	IFN-inducible GTPase 2
AV110584	MARCKS-like protein
M13227	preproenkephalin 1
N M_011315	serum amyloid A 3
N M_007707	suppressor of cytokine signaling 3 (SOCS-3)
AW491448	transcribed locus

b Downregulation of genes (<one third) by OXZ induction and upregulation after saireito administration

BB393498	5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase
BB718075	acetyl-coenzyme A acyltransferase 2 (mitochondrial 3-oxoacyl-coenzyme A thiolase)
AK013376	amyloid-β (A4) precursor-like protein 2
AK018763	angiotensinogen
BF137345	baculoviral IAP repeat-containing-4
BC022605	cAMP responsive element binding protein 3-like 4
NM_007675	CEA-related cell adhesion molecule 10
NM_007694	chromogranin B
NM_016675	claudin 2
AB061276	cytidine monophospho-*N*-acetylneuraminic acid hydroxylase
BC018323	D site albumin promoter-binding protein
BC027538	dynamin 1-like
AF043219	general transcription factor II I
BC027194	Golgi phosphoprotein 3-like
BB773386	hedgehog-interacting protein
BG065877	high-density lipoprotein-binding protein
U29768	Ig κ chain
NM_010584	intelectin a
AU079968	kinesin-2
NM_010795	mannoside acetylglucosaminyltransferase 3
NM_015776	microfibrillar-associated protein 5
AV319507	multimerin 2
AV060116	pancreatic lipase-related protein 2
BG801497	ρ-related BTB domain containing 3
M35669	similar to anti-glycoprotein B of human cytomegalovirus immunoglobulin Vl chain
AI787666	similar to pregastric esterase/similar to BC055815 protein
NM_011330	small chemokine (C-C motif) ligand 11
AI428101	SRY-box containing gene 4
BQ176328	threonyl-tRNA synthetase-like 2
AFFX-TransRec-Mur/X57349_5	transferrin receptor
AK015705	transmembrane 4 superfamily member 9
NM_025458	transmembrane emp24 protein transport domain containing 6
BC024702	transthyretin
BB283676	tripartite motif protein 2
AF131102	xenotropic and polytropic retrovirus receptor 1

of IL-4 and IL-10 was upregulated and that of IFN-γ was downregulated [22]. These previous reports indicate that Th2 but not Th1 immune responses are activated in the colon of OC mice, whereas in the present study, mRNA expression of Th2 cytokines was greatly upregulated and that of Th1 cytokine was slightly increased in the colon of our BALB/c mouse OC model. Iijima et al. have reported [19] that the production of IFN-γ and IL-4 by lamina propria lymphocytes is increased in the C57BL/6 mouse OC model. This discrepancy in Th1 immune responses in the colon of OC mouse models could be explained, in part, by genetic differences in the mouse strain and the different doses of intrarectally administered OXZ.

Furthermore, there are few reports on the cytokine profile in the systemic immune system of OC models. The lamina propria mononuclear cells of the colon and splenocytes produce large amounts of IL-4, IL-5, and IL-13 but not IFN-γ by stimulation of the anti-CD3/anti-CD28 antibody in the C57BL/6 mouse OC model [20]. Similarly, in the spleen of our BALB/c mouse OC model, mRNA expression of IL-4 and IL-10 was upregulated and that of IFN-γ was downregulated, indicating that the Th1/Th2 balance shifts toward Th2 dominance in the spleen of murine OC models.

We have reported that prednisolone (10 mg/kg, p.o.) significantly decreases DAS and CDS in our OC model [23]. Since the anti-inflammatory drug prednisolone, which is commonly used for treating human UC, showed an inhibitory effect in our OC model, we consider that the efficacy of new therapeutic agents for human UC can be appropriately evaluated in our OC model.

Oral administration with saireito caused a marked improvement in our OC model. Saireito is prepared from 12 medicinal herbs with multiple bioactive components, including *Bupleuri radix* [24], *Scutellariae radi*x [25, 26], *Ginseng radix* [27], and *Glycyrrhizae radix* [28]; these medicinal herbs possess anti-inflammatory properties. Saikosaponins [29], baicalin [30], and ginsenoside Rh1 [31] are responsible for the medicinal properties of these medicinal herbs and contribute to their pharmacological effects. It is generally considered that traditional Japanese herbal medicines (e.g. Kampo medicines), including saireito, exert pharmacological effects due to the integrated and/or synergistic actions of their components [32].

In agreement with previous experiments [15, 20, 33], the upregulated cytokines were shown to decrease with the suppression of colitis. Similarly, in the colon of our model, Th2 cytokine expression was markedly downregulated with the suppression of colitis by saireito, while the expression of the Th1 cytokine IFN-γ was further up-

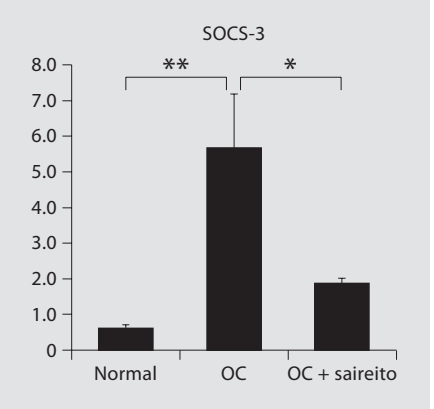

Fig. 3. Expression of SOCS-3 mRNA in OC mice administered saireito. Means ± SEM (normal: n = 7, OC: n = 10, OC + saireito: n = 10, * $p < 0.05$, ** $p < 0.01$).

regulated. Our data demonstrate that saireito administration caused the cytokine profiles to shift toward Th1 dominance and prevented the mucosal immune system from polarizing toward the Th2 environment in the colon of our OC model. Taken together, saireito improved colitis due to its immunomodulatory activities toward Th1 dominance in the mucosal immune system of the colon, but not in the systemic immune system of our OC mice. Saireito has been reported to exert immunomodulatory effects on peripheral blood mononuclear cells [33], decidual cells [34], and splenocytes [35] but not in the intestine. In the spleen of MRL/lpr mouse, a model of systemic lupus nephritis (a Th1-dominant disease), saireito administration increases the production of IL-4 and decreases that of IFN-γ [35]. This discrepancy between this report and our results appears to depend on the type of immune disease and the murine strain used; however, further studies are required for clarification.

Furthermore, it has been reported that patients with retroperitoneal fibrosis were successfully treated with saireito and there was hardly any ureteral obstruction and any other side effect 3 months after the administration of saireito [36]. It is well known that saireito rarely causes side effects such as immunodeficiency, gastroduodenal ulcer and osteoporosis that often accompany long-term administration of corticosteroids. Taken together, it is suggested that saireito appears to exert its immunomodulatory effect specifically on the inflammatory sites and have little effect on non-inflammatory sites, thereby minimizing its immunological side effects.

As mentioned above, Kampo medicines are treatment formulas comprising combinations of medicinal herbs

that contain multiple bioactive components. Various types of immune cells and numerous immunity-related molecules play a complex role in the pathogenesis and development of UC in experimental models as well as in humans; this mechanism of UC pathogenesis is still unclear to the researchers conducting pharmacological and pathophysiological assessments in experimental UC models [14]. Therefore, we performed a global transcriptome analysis with microarray system in order to assess the transcription in the colon of our OC mice. The results provided 47 genes that fulfilled our criteria for candidate colitis-related genes. Most of these genes were associated with the cell cycle, and their functions remain to be elucidated.

However, we found some very interesting genes that were affected in the OC and saireito-treated OC mice (table 2). The MARCKS-like protein gene is specifically expressed in M cells over gut-associated lymphatic tissue (Payer's patches and isolated lymphoid follicles) and elevated in the OC mice and decreased in the saireito-treated OC mice (table 2a), suggesting that the enhanced uptake of luminal antigens by increased or activated M cells may be an important factor in the pathogenesis of OC. Moreover, the expression level of claudin 2 mRNA was downregulated in the OC mice and was restored by saireito. The enhanced tight junction permeability in the epithelium of the OC mouse colon may cause the enhanced uptake of luminal antigens. Therefore, saireito exerted the inhibitory effect on OC via the prevention of luminal antigen uptake in the OC mouse colon.

Serum amyloid A is one of the acute-phase proteins induced by IL-1 and TNF during inflammation. Saireito suppressed enhanced expression of serum amyloid A mRNA, revealing the anti-inflammatory effect of saireito in the OC model.

Most interestingly, the microarray analysis demonstrated that SOCS-3 mRNA expression that was upregulated in the colon of our OC mice was decreased after saireito treatment. SOCS proteins are negative feedback inhibitors of cytokine signaling transduction, which potently block the Janus kinase/signal transducers and activators of transcription (Jak/STAT) pathway and participate in the pathogenesis of various inflammatory diseases [37]. In T-helper cells, SOCS-3 is predominantly expressed in Th2 cells and negatively regulates the Th1 pathway from IL-12 to STAT-4 [38]. Therefore, SOCS-3 is considered to play an important role in regulating the onset and development of Th2-mediated immune diseases, atopic dermatitis [39], and asthma [40]. In addition, it has been reported that SOCS-3 expression was increased and remained increased for 2 weeks in a mouse dextran sulfate-induced colitis model [41], and that SOCS-3 protein was increased and localized primarily in lamina propria lymphocytes with comparatively lower expression in the crypt epithelial cells in a murine model of IL-10 deficiency-induced colitis [42].

Further SOCS-3 mRNA expression was analyzed with real-time PCR, which clearly revealed the changes in our OC mice. In addition, the OC mouse model used in this study is a suitable mouse model that resembles human UC. Our findings confirm a previous study reporting a good correlation between the level of mucosal SOCS-3 expression and the degree of both endoscopic and histological inflammation in human UC [43].

In conclusion, we established a modified OC model that resembles human UC, in which the systemic and mucosal immune system was shifted toward Th2 dominance and disclosed a novel mechanism of the suppression of upregulated SOCS-3, one of the mechanisms by which saireito exerts its inhibitory effect. Our results provide experimental evidence for many clinical reports on the amelioration of symptoms associated with human UC following saireito treatment. Further, our results support the therapeutic effect of saireito on human UC. Taken together, our results indicate that saireito has therapeutic potential as a novel strategy for controlling human UC worldwide.

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References

1 Podolsky DK: Inflammatory bowel disease. N Engl J Med 2002;347:417–429.

2 Baumgart DC, Carding SR: Inflammatory bowel disease: cause and immunobiology. Lancet 2007;369:1627–1640.

3 Hibi T, Ogata H: Novel pathophysiological concepts of inflammatory bowel disease. J Gastroenterol 2006;41:10–16.

4 Kanis JA, Johansson H, Oden A, Johnell O, de Laet C, Melton LJ III, Tenenhouse A, Reeve J, Silman AJ, Pols HA, Eisman JA, McCloskey EV, Mellstrom D: A meta-analysis of prior corticosteroid use and fracture risk. J Bone Miner Res 2004;19:893–899.

5 James ER: The etiology of steroid cataract. J Ocul Pharmacol Ther 2007;23:403–420.
6 Deshmukh CT: Minimizing side effects of systemic corticosteroids in children. Indian J Dermatol Venereol Leprol 2007;73:218–221.
7 Baumgart DC, Sandborn WJ: Inflammatory bowel disease: clinical aspects and established and evolving therapies. Lancet 2007; 369:1641–1657.
8 Li FX, Verhoef MJ, Best A, Otley A, Hilsden RJ: Why patients with inflammatory bowel disease use or do not use complementary and alternative medicine: a Canadian national survey. Can J Gastroenterol 2005;19:567–573.
9 Fujimoto M, Mori A, Sekiya N, Shimada Y, Terasawa K: Two cases of ulcerative colitis successfully treated with Kigi-kenchu-to (in Japanese). Jpn J Oriental Med 2004;55:655–660.
10 Ohno S: Roles of Kampo medicine in treating rheumatic diseases. J Trad Med 2007;24: 73–80.
11 Kimura K, Nanba S, Tojo A, Matsuoka H, Sugimoto T: Effects of sairei-to on the relapse of steroid-dependent nephrotic syndrome. Am J Chin Med 1990;18:45–50.
12 Matsuike T, Yasumoto S, Nozawa H, Nishino H, Matsushima M, Kamakura H, Watanabe Y, Matsushima Y: Significance of treatment with Kampo medicines for ulcerative colitis. Prog Med 1999;19:879–885.
13 Okubo A, Masuda H, Hayashi S, Hayashi I, Yamagishi M, Aoki H, Taniguchi T, Kato K: Clinical study of Kampo-medicine (saireito) for ulcerative colitis. J Nihon Univ Med Assoc 1996;55:286–289.
14 Strober W, Fuss I, Mannon P: The fundamental basis of inflammatory bowel disease. J Clin Invest 2007;117:514–521.
15 Boirivant M, Fuss IJ, Chu A, Strober W: Oxazolone colitis: a murine model of T helper cell type 2 colitis treatable with antibodies to interleukin 4. J Exp Med 1998;188:1929–1939.
16 Bhan AK, Mizoguchi E, Smith RN, Mizoguchi A: Colitis in transgenic and knockout animals as models of human inflammatory bowel disease. Immunol Rev 1999;169:195–207.
17 Wirtz S, Neufert C, Weigmann B, Neurath MF: Chemically induced mouse models of intestinal inflammation. Nat Protoc 2007;2: 541–546.
18 Kawada M, Arihiro A, Mizoguchi E: Insights from advances in research of chemically induced experimental models of human inflammatory bowel disease. World J Gastroenterol 2007;13:5581–5593.
19 Iijima H, Neurath MF, Nagaishi T, Glickman JN, Nieuwenhuis EE, Nakajima A, Chen D, Fuss IJ, Utku N, Lewicki DN, Becker C, Gallagher TM, Holmes KV, Blumberg RS: Specific regulation of T helper cell 1-mediated murine colitis by CEACAM1. J Exp Med 2004;199:471–482.
20 Heller F, Fuss IJ, Nieuwenhuis EE, Blumberg RS, Strober W: Oxazolone colitis, a Th2 colitis model resembling ulcerative colitis, is mediated by IL-13-producing NK-T cells. Immunity 2002;17:629–638.
21 Ishii M, Hashimoto S, Tsutsumi S, Wada Y, Matsushima K, Kodama T, Aburatani H: Direct comparison of GeneChip and SAGE on the quantitative accuracy in transcript profiling analysis. Genomics 2000;68:136–143.
22 Cenac N, Cellars L, Steinhoff M, Andrade-Gordon P, Hollenberg MD, Wallace JL, Fiorucci S, Vergnolle N: Proteinase-activated receptor-1 is an anti-inflammatory signal for colitis mediated by a type 2 immune response. Inflamm Bowel Dis 2005;11:792–798.
23 Watanabe T, Yamamoto T, Yoshida M, Fujiwara K, Aburatani H, Shimada Y, Kadowaki M: Therapeutic effects of saireito, a traditional herbal medicine, in oxazolone induced mouse ulcerative colitis via the suppression of enhanced expression of the suppressor of cytokine signaling (SOCS)-3. Gastroenterology 2007;132:A-394.
24 Fuse S, Shiotani Y, Shimada T, Terasawa K, Sagara K: Studies on the anti-inflammatory effects of the medicinal plant 'Saiko' (*Bupleurum falcatum* L.). J Trad Med 1994;11: 206–213.
25 Choi YA, Kang OH, Park HJ, Tae J, Kim DK, Kang CS, Choi SC, Yun KJ, Choi SJ, Nah YH, Kim YH, Bae KH, Lee YM: Effect of processed *Scutellaria baicalensis* on dextran sulfate sodium-induced colitis in mice. Int J Mol Med 2005;16:667–672.
26 Chung HL, Yue GG, To KF, Su YL, Huang Y, Ko WH: Effect of *Scutellariae radix* extract on experimental dextran-sulfate sodium-induced colitis in rats. World J Gastroenterol 2007;13:5605–5611.
27 Hofseth LJ, Wargovich MJ: Inflammation, cancer, and targets of ginseng. J Nutr 2007; 137:183S–185S.
28 Yuan H, Ji WS, Wu KX, Jiao JX, Sun LH, Feng YT: Anti-inflammatory effect of diammonium glycyrrhizinate in a rat model of ulcerative colitis. World J Gastroenterol 2006;12: 4578–4581.
29 Ushio Y, Oda Y, Abe H: Effect of saikosaponin on the immune responses in mice. Int J Immunopharmacol 1991;13:501–508.
30 Hong T, Jin GB, Cho S, Cyong JC: Evaluation of the anti-inflammatory effect of baicalein on dextran sulfate sodium-induced colitis in mice. Planta Med 2002;68:268–271.
31 Park EK, Choo MK, Han MJ, Kim DH: Ginsenoside Rh1 possesses antiallergic and anti-inflammatory activities. Int Arch Allergy Immunol 2004;133:113–120.
32 Borchers AT, Hackman RM, Keen CL, Stern JS, Gershwin ME: Complementary medicine: a review of immunomodulatory effects of Chinese herbal medicines. Am J Clin Nutr 1997;66:1303–1312.
33 Yamashiki M, Nishimura A, Watanabe J, Nakano T, Kosaka Y: Effects of the herbal medicine 'Sai-rei-to' on in vitro interferon-gamma production of peripheral blood mononuclear cells. J Clin Lab Immunol 1992;38:153–160.
34 Fujii O, Kanai T, Kouzuma S, Baba K, Miki A, Hyodo H, Yamashita T, Unno N, Taketani Y: Herbal medicines, Sairei-to and Tokishakuyaku-san, differently modulate the release of cytokines from decidual versus peripheral blood mononuclear cells. Am J Reprod Immunol 2001;46:369–372.
35 Ito T, Seo N, Yagi H, Ohtani T, Tokura Y, Takigawa M, Furukawa F: Unique therapeutic effects of the Japanese-Chinese herbal medicine, Sairei-to, on Th1/Th2 cytokines balance of the autoimmunity of MRL/lpr mice. J Dermatol Sci 2002;28:198–210.
36 Asano T, Fujii Y, Numao N, Kageyama Y, Kihara K: The efficiency of Sairei-to for retroperitoneal fibrosis: two case reports. Hinyokika Kiyo 2006;52:543–547.
37 Alexander WS, Hilton DJ: The role of suppressors of cytokine signaling (SOCS) proteins in regulation of the immune response. Annu Rev Immunol 2004;22:503–529.
38 Yamamoto K, Yamaguchi M, Miyasaka N, Miura O: SOCS-3 inhibits IL-12-induced STAT4 activation by binding through its SH2 domain to the STAT4 docking site in the IL-12 receptor β2 subunit. Biochem Biophys Res Commun 2003;310:1188–1193.
39 Ekelund E, Saaf A, Tengvall-Linder M, Melen E, Link J, Barker J, Reynolds NJ, Meggitt SJ, Kere J, Wahlgren CF, Pershagen G, Wickman M, Nordenskjold M, Kockum I, Bradley M: Elevated expression and genetic association links the SOCS3 gene to atopic dermatitis. Am J Hum Genet 2006;78:1060–1065.
40 Seki Y, Inoue H, Nagata N, Hayashi K, Fukuyama S, Matsumoto K, Komine O, Hamano S, Himeno K, Inagaki-Ohara K, Cacalano N, O'Garra A, Oshida T, Saito H, Johnston JA, Yoshimura A, Kubo M: SOCS-3 regulates onset and maintenance of T(H)2-mediated allergic responses. Nat Med 2003;9:1047–1054.
41 Suzuki A, Hanada T, Mitsuyama K, Yoshida T, Kamizono S, Hoshino T, Kubo M, Yamashita A, Okabe M, Takeda K, Akira S, Matsumoto S, Toyonaga A, Sata M, Yoshimura A: CIS3/SOCS3/SSI3 plays a negative regulatory role in STAT3 activation and intestinal inflammation. J Exp Med 2001; 193:471–481.
42 Han X, Sosnowska D, Bonkowski EL, Denson LA: Growth hormone inhibits signal transducer and activator of transcription 3 activation and reduces disease activity in murine colitis. Gastroenterology 2005;129: 185–203.
43 Miyanaka Y, Ueno Y, Tanaka S, Yoshioka K, Hatakeyama T, Shimamoto M, Sumii M, Chayama K: Clinical significance of mucosal suppressors of cytokine signaling 3 expression in ulcerative colitis. World J Gastroenterol 2007;13:2939–2944.