Incentive Award, 2010

Current status of basic research in Kampo therapy using a murine colitis

Mari Endo, a) Tetsuro Oikawa, a) Tsutomu Hatori, a) Tsukasa Matsumoto, a) Toshihiko Hanawa a), b)

a) Department of Clinical Research, Oriental Medicine Research Center, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8642, Japan. b) Kitasato University Graduate School, Doctoral Program of Medical Science, Oriental Medicine, 1-15-1 Kitasato, Sagamihara, Kanagawa 228-8555, Japan. c) Department of Surgical Pathology, Toho University Omori Medical Center, 6-11-1 Omori nishi, Ota-ku, Tokyo 143-8541, Japan. d) Department of Pharmacy, Faculty of Pharmacy, Iwaki Meisei University, 5-5-1 Chuodai, Iino, Iwaki, Fukushima 970-8551, Japan. (Accepted August 4, 2011.)

Abstract

Kampo medications have been successfully used for patients with ulcerative colitis (UC) who have demonstrated difficulty in adjusting to conventional Western pharmacological therapy. These patients have developed serious adverse drug reactions and have shown little improvement or an actual decrease in Quality of Life (QOL) in spite of the pharmacological intervention. Kampo medications, on the other hand, can increase QOL in such patients. However, few reports are available that detail the effectiveness and/or mechanism(s) of action of Kampo medications for the treatment of UC.

In this article, the characteristics of chemically-induced murine colitis are reviewed. Chemically-induced murine colitis is the most commonly employed experimental animal model of colitis and shares important characteristics with UC. Recent research regarding the administration and anti-inflammatory actions of Kampo medications for the management of experimental murine colitis, while limited, is also reviewed. In addition, the importance of herb combinations (as opposed to any single herb) that comprise Kampo formulations are described in the context of research from this laboratory. This work demonstrates that the herbs in Kampo medications may act synergistically to protect against dextran sulfate sodium (DSS)-induced murine colitis. Finally, the applicability of this work to the treatment of UC in human patients is discussed.

Key words ulcerative colitis, experimental murine model of colitis, “minus one herb” formulation.

Introduction

Ulcerative colitis (UC) is a chronic, recurrent inflammatory disease of the large intestine that is characterized by abdominal pains, bloody diarrhea, fever, anemia, anorexia, loss of body weight, intestinal mucosal ulceration and infiltration of neutrophils and lymphocytes in the mucous membranes. UC has been designated as a specific disease by the Ministry of Health, Labour and Welfare of Japan. Teenagers and young adults are at the highest risk for the development of UC. Although the etiology of US is still unknown, the condition appears to stem from interactions between genetic, immunological and environmental factors that result in an abnormal immune response in the intestinal mucosa to certain intraluminal antigens.1)

Recent surveys have indicated that the number of patients diagnosed with UC is growing each year in Japan (Figure 1). The disease is intermittent in nature;
however, patients with UC are prone to frequent relapse, and the disease becomes chronic in most patients. While conventional Western pharmacological therapy (e.g., treatment with corticosteroids and/or immunosuppressive drugs) is effective against UC, this therapy in and of itself is not helpful infrequently and may in fact be harmful for some UC patients due to the appearance of adverse drug reactions. Some UC patients that suffer from relatively mild inflammation as indicated by endoscopy have reported a decrease in the Quality of Life (QOL) following standard pharmacologic intervention, with a corresponding increase in subjective symptoms such as abdominal pain.

Kampo medications (Japanese herbal medications) have been used as a complementary or alternative QOL-improving treatment for UC patients who do not respond well to conventional Western pharmacological therapy. However, few reports are available regarding the efficacy of this complementary UC therapy in human patients. As such, basic research employing animal models of experimental colitis is anticipated to provide valuable information that will be critical for the application of Kampo medications to the clinical setting. On the other hand, reports detailing the efficacy of Kampo medicine in experimental animal models of colitis are also limited.

This article reviews the current research that describes the inhibitory actions of Kampo medicine against experimental colitis in a variety of animal models. Next, work from this laboratory is introduced that demonstrates the anti-inflammatory activity of Kampo medications against dextran sulfate sodium (DSS)-induced murine colitis. Furthermore, combinations of herbal constituents as opposed to single herbs that comprise Kampo formulations are shown to be essential for the most efficacious anti-inflammatory activity against DSS-induced murine colitis.

1) Experimental animal models of colitis

1) Chemically-induced colitis models

Experimental animal models of colitis are invaluable for the analysis of the pathophysiology of inflammatory bowel disease including UC and the mechanisms of action of numerous new therapeutic drugs. Experimental colitis models are classified into predominant categories based on the method of disease induction, as shown in Table 1.12) Of these, chemically-induced murine colitis is the most commonly used experimental animal model of colitis. The primary features of chemically-induced murine colitis are discussed in references13–18) and summarized in Table 2. Specific chemically-induced colitis models are discussed below.

a) Carrageenan-induced colitis model

Carrageenan is a naturally occurring sulfated polysaccharide derived from seaweed. Following administration of carrageenan to mice, the compound is taken up by the macrophages in the colon. This leads to the activation of lysosomes and an increase in intracellular enzymes. Although the precise pathophysiological mechanism of carrageenan-induced murine colitis remains unclear, impairment of the mucosal barrier in epithelial cells due to the increase in enzyme levels appears to be a causative factor.19)

b) DSS-induced colitis model

DSS is a long chain glucose polymer with inflammatory properties in the digestive tract. The histological and pathological characteristics of DSS-induced experimental murine colitis resemble those of UC in humans. However, as for carrageenan-induced colitis, the pathogenic mechanism of DSS-induced murine colitis is yet to be elucidated. Nonetheless, the ad libitum administration of DSS-infused drinking water to mice is sufficient to cause hematochezia, loss of body weight, shortening of the colon, mucosal ulcers and infiltration of neutrophils. In addition, DSS is directly toxic to intestinal epithelial cells of the basal crypts and affects the
Table 1 Experimental colitis models.

<table>
<thead>
<tr>
<th>Genetically engineered models</th>
<th>Chemical-induced model</th>
<th>Adoptive transfer model</th>
<th>Spontaneous model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interleukin-2 KO/IL-2 receptor (R)α KO mice</td>
<td>IL-7 transgenic mice</td>
<td>Acetic-acid-induced colitis</td>
<td>C3H/HeJ/Bir mice</td>
</tr>
<tr>
<td>IL-10 KO mice</td>
<td>Signal transducer and activating transcription (STAT)-4 transgenic mice</td>
<td>Iodoacetamide-induced colitis</td>
<td>CD45RB transfer model</td>
</tr>
<tr>
<td>T cell receptor mutant mice</td>
<td>OX40 ligand-transgenic mice</td>
<td>Indomethacin-induced enterocolitis</td>
<td>SAMP1/Yit mice</td>
</tr>
<tr>
<td>TNF-3° untranslated region KO mice</td>
<td></td>
<td>TNBS-induced colitis</td>
<td></td>
</tr>
<tr>
<td>Trefoil factor-deficient mice</td>
<td></td>
<td>Oxazolone-induced colitis</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>DSS-induced colitis</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Peptidoglycan-polysaccharide(PG-PS)-induced colitis</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Carageenan-induced colitis</td>
<td></td>
</tr>
</tbody>
</table>

d) Oxazolone-induced colitis model

Oxazolone, like TNBS, is a haptenizing agent used to induce experimental colitis. High-dose oxazolone by rectal administration induces a delayed hypersensitivity colitis response in mice that is mediated by T-helper type-2 (Th2) cells. On the other hand, low-dose oxazolone can induce a mixed Th1/Th2-dependent colitis. As with TNBS-induced colitis, the oxazolone-induced colitis model is advantageous for the analysis of antigen-specific immune responses.

2) Shortcomings of chemically-induced murine models of colitis

All of the above chemically-induced murine models of colitis share important immunological and histopathological aspects with the UC disease that afflicts human patients. However, murine models of colitis also differ in critical ways from UC in humans. Shortcomings of chemically-induced murine models of colitis are briefly discussed below, including limitations of their use for the study of the efficacy of anti-inflammatory agents for the treatment of UC.

a) Carageenan-induced murine colitis model

The inflammation associated with carageenan-induced murine colitis is less chronic than that associ-
Table 2 The primary features of chemically-induced colitis models.

<table>
<thead>
<tr>
<th>chemicals</th>
<th>administration route</th>
<th>animal species</th>
<th>location of mucosal inflammation</th>
<th>occurrence of colitis</th>
<th>continuous of colitis after removal of the chemicals</th>
<th>site of action</th>
<th>possible mechanism of action</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong></td>
<td>drinking water</td>
<td>guinea pigs</td>
<td>cecum</td>
<td>1 week</td>
<td>1-2 weeks</td>
<td>epithelial cells (macrophage)</td>
<td>anaerobic bacteria (in particular, bacteroides spp.) \ deviation of lysosomal enzyme by increased and hypertrophy macrophage \ initial action of occurrence of inflammation: PGE2 \ continuation of inflammation: LTB4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>mice</td>
<td>left side of the colon</td>
<td>within 3-6 weeks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>rabbits</td>
<td>whole large intestine</td>
<td>7 weeks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>B</strong></td>
<td>drinking water</td>
<td>hamsters</td>
<td>distal colon</td>
<td>1 week</td>
<td></td>
<td>epithelial cells (macrophage)</td>
<td>increasing of gram-negative anaerobic bacteria \ deviation of lysosomal enzyme by increased and hypertrophy macrophage \ T-cell and B-cell</td>
</tr>
<tr>
<td></td>
<td></td>
<td>guinea pigs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>rabbits</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>BALB/c mice</td>
<td>left side of the colon</td>
<td>5-7 days</td>
<td>1 week</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C57BL/6 mice</td>
<td></td>
<td>5-7 days</td>
<td>2 week</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>C</strong></td>
<td>enema administration</td>
<td>mice</td>
<td>exposed area of the colon</td>
<td>3-5 days</td>
<td>1 week</td>
<td>macrophage</td>
<td>immune response to trinitrophenylation autoantigen \ T helper cell-dependent mucosal immune response \ CD4+ T cell</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rat rabbits</td>
<td></td>
<td></td>
<td></td>
<td>lymphocyte</td>
<td>IL-12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SJL/J mice</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IFN-γ IL-2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BALB/c mice</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>IFN-γ−/− mice</td>
<td>(Balb/c background)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>D</strong></td>
<td>enema administration</td>
<td>mice</td>
<td>localized in the distal colon</td>
<td>2-5 days</td>
<td>9 days</td>
<td>lamina propria lymphocytes</td>
<td>elevated productions Th2 cytokines \ increased IL-4 and IL-5, but not IFN-γ \ (at lower doses) \ mixed Th1/Th2 dependent colitis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A: Carrageenan colitis, B: DSS colitis, C: TNBS colitis, D: Oxazolone colitis
CA: degraded carrageenan polymers (sulfate polysaccharide), DSS: dextran sulfate sodium (sulfated polysaccharide), TNBS: trinitrourbenezene sulfonic acid, OX: oxazolone

ated with UC in human patients. Furthermore, the inflammation is localized to the cecal area in mice, whereas inflammation is widespread and observed from the proximal to distal colon in human patients with UC. **b) DSS-induced murine colitis model**

DSS effects are mouse strain-dependent. For example, C57BL/6 mice are more sensitive to the colitis-inducing actions of DSS than are BALB/c mice. However, it is unclear whether C57BL/6 mice or BALB/c mice provide a more appropriate model for the study of the response to anti-inflammatory drugs that are used for the treatment of UC in humans.  

**c) TNBS-induced murine colitis model**

TNBS has a narrow concentration range for the induction of colitis because the lethal dose range is similar to that of the inductive dose range. Moreover, TNBS-induced murine colitis is susceptible to environmental influences in the laboratory, which can either increase or decrease the severity of the condition. In addition, like DSS-induced colitis, the severity of TNBS-induced colitis is highly dependent on the mouse strain, and strain-dependent optimization of the TNBS dosage is required.  

**d) Oxazolone-induced murine colitis model**

Oxazolone-induced murine colitis is also strain-dependent and, like TNBS, strain-dependent optimization of the oxazolone dosage is required.
II) Anti-inflammatory actions of Kampo medications in experimental animal models of colitis and related concerns

1) The choice of single herb medications versus herb combinations

Kampo medications are composed of various combinations of herbs and associated herbal compounds. They are usually administered orally for the treatment of human disease. Their therapeutic actions are thought to be due to the activity of mixtures of the original herbal compounds as well as their metabolites. Therefore, basic research conducted in whole animals with single herbs as well as combinations of herbs will be crucial for understanding the mechanisms of action of Kampo medications and their byproducts. As noted above, experimental animal models of colitis are expected to be useful for the in vivo study of both the pathophysiology of UC and the therapeutic efficacy of newly-developed drug therapies, including Kampo therapies. However, scant information is actually available regarding the effectiveness of Kampo medications in such experimental animal models of colitis. Kampo medications that do have reported anti-inflammatory activity against experimental animal colitis are shown in Table 3. These include orengedokuto, hangeshashinto and saireito.

Orengedokuto together with Scutellariae Radix and baicalein, the main components of orengedokuto, have been reported to prevent or inhibit DSS-induced murine colitis. However, no reports are available regarding the effects of other Kampo medications for the treatment of DSS-induced animal colitis. Therefore, the anti-inflammatory actions of various additional Kampo medications including formulations which seem to be unrelated with a direct anti-inflammatory effect in the treatment against DSS-induced murine colitis were investigated, resulting in the demonstration of anti-inflammatory activity of saireito by this group (see below). In another animal model, experimental colitis in rats induced by intracolonic administration of TNBS was shown to be ameliorated by the anti-inflammatory effects of hangeshashinto. The therapeutic actions of hangeshashinto may have been largely due to the mixture of glycyrrhizin derived from Glycyrrhizae Radix and ginsenosides, the saponin fraction of Ginseng Radix. However, it is not yet clear from these reports whether a single herb alone or a particular combination of herbal components was responsible for mediating the efficacy of the Kampo medications. In particular, no information is available regarding the effects of Kampo medications that do not contain Scutellariae Radix.

The hypothesis was therefore evaluated that Kampo formulations “minus one herb” are useful for the analysis of the therapeutic impact of specific herbs. These “minus one herb” formulations were employed in an attempt to determine which herbs are crucial for the efficacy of Kampo medications against DSS-induced murine colitis. As described below, herbal combinations as opposed to single herbs were found to be essential for the optimal anti-inflammatory activity of Kampo medications.

2) Administration schedule of Kampo medicine and DSS in DSS-induced murine colitis

For the study of the efficacy of anti-inflammatory agents against DSS-induced murine colitis, it is desirable to set an administration schedule of Kampo medicine after the onset of colitis so as to better reflect the clinical situation. Recently reported drug and DSS administration schedules are summarized below. As shown in Figure 2, drugs can be administered prior to, concurrently with, or after DSS in murine models of colitis. In work from our laboratory (described below), mice were treated simultaneously with Kampo medications and DSS. However, the best timing remains to be established for the most accurate simulation of drug therapy in the clinical setting.

III) Elucidation of the importance of herbal combinations in Kampo formulations

1) Current reports on the efficacy of saireito against assorted diseases

Kampo medicine has been shown to be effective even in the case of patients that are afflicted with two or more different diseases. For example, saireito has been used for the treatment of oliguria, edema, vomiting and diarrhea, assorted nephritic diseases and connective tissue disease in addition to inflammatory bowel disease. This section focuses on the actions of saireito and its constituents, shosaikoto and gorieisan, against conditions
Ginseng Radix and Zizyphi Fructus). Second, saireito, goreisan and shosaikoto were found to inhibit lipid hydroperoxide-induced corneal neovascularization and to decrease levels of corneal vascular endothelial growth factors thought to play a role in neovascularization in the rabbit cornea, indicating the involvement of a common herb or herbs to saireito, goreisan and shosaikoto. Third, goreisan showed inhibitory effects against the degranulation of murine bone marrow-derived mast cells (mBMMCs) in a dose-dependent manner. Among the five constituent medicinal herbs of goreisan, both Poria and Polyergus had inhibitory activity against mBMMCs. Ergosterol, a principal and common chemical component of Poria and Polyergus, also suppressed the degranulation of mBMMCs. Fourth, saireito-induced hyporesponsiveness to cardiac allografts in a dose-dependent manner in a murine model of cardiac transplantation with fully mismatched allografts. However, none of the single crude drug extracts of saireito, shosaikoto or goreisan prolonged allograft survival, suggesting that the clinical effects of saireito required administration of all of its constituent herbs.

2) Activity of Kampo medications against DSS-induced murine colitis

Research from this group is presented herein that demonstrates the activity of the Kampo medications oregedokuto and saireito against DSS-induced murine colitis.

a) Screening of Kampo medications with anti-inflammatory properties against DSS-induced murine colitis

Kampo lyophilized powder produced by this group was screened for a suppressor of DSS-induced colon shortening, myeloperoxidase (MPO) activity and weight loss in mice. Thirteen Kampo medications were initially selected. These included oregedokuto, saireito, hokuekkito, koso, hangekokoboku, shimoto, ryokei-jutsuko, yokokusan, keishibukuryogan, maoto, daijokoto, juzentaihoto and sigyakuto. Previous work showed that, of these, saireito and oregedokuto inhibited DSS-induced murine colitis. The demonstrated anti-inflammatory activity of oregedokuto is in agreement with research from Hong et al. (Table 3), which showed that oregedokuto was as effective as salazosulfapyridine against DSS-induced colitis.
Table 3 Anti inflammatory actions of Kampo medicines in experimental animal models of colitis.

<table>
<thead>
<tr>
<th>Author</th>
<th>Experimental Strain</th>
<th>animal colitis inducing agent</th>
<th>Kampo medicine</th>
<th>Outline of study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hong et al.</td>
<td>BALB/c mice</td>
<td>DSS</td>
<td>Orendokuto</td>
<td>restored the lost body weight. increased the hemoglobin content. Decreased the degree of inflammation. improved the histological signs of inflammation. Increased the LFA-1 expression on CD3+ cell. decreased the LFA-1 expression on other cell.</td>
</tr>
<tr>
<td>Hong et al.</td>
<td>BALB/c mice</td>
<td>DSS</td>
<td>Ogon</td>
<td>restored the lost body weight. increased the hemoglobin content. Decreased the degree of inflammation. improved the histological signs of inflammation.</td>
</tr>
<tr>
<td>Hong et al.</td>
<td>BALB/c mice</td>
<td>DSS</td>
<td>baicalein</td>
<td>Ameliorates all the considered inflammatory symptoms of the induced colitis, such as body weight loss, blood haemoglobin content, rectal bleeding and other histological and biochemical parameters.</td>
</tr>
<tr>
<td>Kawashima et al.</td>
<td>Wistar/ST rats</td>
<td>TNBS</td>
<td>Hangeshashinto</td>
<td>reduced the colonic damage</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>BA+BE+GL+GS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>BA+BE</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>GL+GS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>BA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>BE</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>GL</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>GS</td>
</tr>
<tr>
<td>Watanabe et al.</td>
<td>BALB/c mice</td>
<td>oxazolone</td>
<td>Saireito</td>
<td>ameliorated colitis enhanced expression of Th2 cytokine mRNAs markedly down regulated with that of IFN-γ mRNA was furter upregulated no effect on the transcription of either type of cytokine in the spleen greatly down regulated the enhanced expression of the suppressor of cytokine signaling-3 mRNA</td>
</tr>
</tbody>
</table>

DSS; dextran sulfate sodium  
TNBS; 2,4,6-trinitrobenzene sulfonic acid  
BA; baicalin  single component of Coptis Rhizome in orendokuto and hangeshashinto  
BE; berberine  single component of Scutellaria Root in hangeshashinto  
GL; glycyrrhizin  single component of Glycyrrhiza in hangeshashinto  
GS; ginsenosides  single component of Ginseng in hangeshashinto

b) Suppression of DSS-induced murine colitis by orendokuto

Scutellariae Radix and Coptis Rhizoma are thought to play key roles in Kampo medications that are used for the treatment of inflammatory diseases, including UC. However, no information is available regarding the effects of Kampo medications that do not include Scutellariae Radix and/or Coptis Rhizoma. Because orendokuto is a formulation that includes both Scutellaria Radix and Coptis Rhizoma, the question arises as to whether orendokuto retains anti-inflammatory activity in the absence of these herbal
constituents. The components of orengedokuto are listed in Figure 3. The anti-inflammatory activity of orengedokuto against DSS-induced murine colitis was mostly lost after the removal of Scutellariae Radix (Figure 4), consistent with results published by Hong et al. (Table 3). Hence, as previously reported, Scutellariae Radix may be the primary active component in orengedokuto.

c) Suppression of DSS-induced murine colitis by saireito

Saireito is composed of two Kampo medications, shosaikoto and goreisan, and is used to treat immune-related diseases. The components of saireito are listed in Figure 3. The anti-inflammatory activities of shosaikoto and goreisan alone were first investigated. As shown in Figure 5, neither shosaikoto nor goreisan by itself showed significant anti-inflammatory activity against DSS-induced murine colitis. Therefore, both components together are essential for the actions of saireito. The herbal component Bupleuri Radix is thought to exert significant modulatory effects on the immune system, although to date no information is available regarding its therapeutic impact on UC. Because saireito includes both Scutellariae Radix and Bupleuri Radix, the retention of anti-inflammatory activity by saireito in the absence of Scutellariae Radix or Bupleuri Radix was next evaluated. Contrary to the findings with orengedokuto (Figure 4), saireito depleted of Scutellariae Radix did not differ from saireito in its anti-inflammatory activity (Figure 6). However, the anti-inflammatory effect of saireito was mostly lost in the absence of Bupleuri Radix, although no inhibition of DSS-induced murine colitis without specific herbs on changes in colon length, MPO activity and colonic damage in mice.

A; Effects of OGT without specific herb on the change of the colon length in mice with colitis induced by DSS.
B; Effects of OGT without specific herb on MPO activity of inflamed distal colon wet tissue in mice.
C; Effects of OGT without specific herb on histological evaluation of inflamed colon in mice. (Control, DSS-induced colitis; OGT, OGT-agon and OGT-oren, The group administrated orengedokuto, orengedokuto without Scutellariae Radix and orengedokuto without Coptidis Rhizome on DSS-induced colitis.) Results are indicated as the mean ± S.E. (n = 5). Significant difference at p < 0.05; * compared with DSS-induced colitis group, # compared with normal group.
Figure 5  Effects of shosaikoto and goreisan on change in colon length, MPO activity and body weight in mice. A: Effects of SST and GRS on the change of colon length in mice with colitis induced by DSS. B: Effects of SST and GRS on MPO activity in inflamed colonic tissues. C: Effects of SST and GRS on body weight in mice with colitis induced by DSS. Abbreviations (Control, DSS-induced colitis; SST, SST and GRS, The group administrated saireito, shosaikoto and goreisan on DSS-induced colitis. ◊; Normal ■; Control ○; SRT △; SST □; GRS.) Results are indicated as the mean ± S.E. (n = 5). Significant difference at p < 0.05; *compared with DSS-induced colitis group, #compared with normal group.

Figure 6  Effects of saireito without specific herbs on change in colon length, MPO activity and colonic damage in mice. A: Effects of SRT without specific herb on the change of the colon length in mice with colitis induced by DSS. B: Effects of SRT without specific herb on MPO activity of inflamed distal colon wet tissue in mice. C: Effects of SRT without specific herb on histological evaluation of inflamed colon in mice. Abbreviations (Control, DSS-induced colitis; SRT, SRT-ogon and SRT-saiko, The group administrated saireito, saireito without Scutellariae Radix and saireito without Bupleuri Radix on DSS-induced colitis.) Results are indicated as the mean ± S.E. (n = 5). Significant difference at p < 0.05; *compared with DSS-induced colitis group, #compared with normal group.
colitis was shown with shosaikoto. Hence, this work demonstrates the importance of shosaikoto and goreisan in combination with Bupleuri Radix for the efficacy of saireito.

d) Summary of the DSS-induced murine colitis study

This work found that i) saireito can suppress DSS-induced murine colitis, ii) Bupleuri Radix may be the principal herbal constituent that contributes to the efficacy of saireito, and iii) the effectiveness of Kampo medications for the treatment DSS-induced murine colitis cannot be attributed to a single herbal constituent such as Scutellariae Radix or Bupleuri Radix. Rather, the combination of all herbs in the Kampo formulation appears to be essential for the most effective anti-inflammatory activity against DSS-induced murine colitis.

The following issues remain to be addressed. First, it will be important to study the efficacy of single constituent herbs as well as herb combinations using Kampo medications in addition to orangedokuto and saireito. This information will be essential for the applicability of Kampo medications to the therapy of UC in future clinical settings.

Second, BALB/c mice were treated simultaneously with Kampo medications and DSS in the current study. It is unknown whether this schedule of Kampo medication administration best reflects the clinical situation. Work is now underway to establish the most appropriate course of drug treatment after the onset of colitis and to optimize the mouse strain employed in further experimental models of DSS-induced murine colitis.

Conclusions

Chemically-induced experimental murine models of colitis resemble UC in humans in certain important immunological and histopathological aspects. However, these colitis models also differ in critical ways from UC in humans. For example, the methods and schedules of administration of Kampo medications in experimental animal models do not necessarily reflect drug administration in the clinical setting. As such, these colitis models must be improved to more accurately to emulate clinical situations, allowing for more precise analysis of the efficacy of Kampo treatments. Finally, the combinations of herbs that comprise Kampo formulations were shown to be essential for effective anti-inflammatory activity against DSS-induced murine colitis. This finding has important implications for the applicability of Kampo therapy to human medicine.

Acknowledgments

This work was supported in part by a Grant-in-Aid for Young Scientists (B), KAKENHI (20790481), provided by The Ministry of Education, Culture, Sports, Science and Technology (MEXT) of the Japanese government.

References

2006. (in Japanese)
responses in the mucosal immune system of the colon. 


30) Shimizu, K., Amagaya, S. and Ogihara, Y.: Structural 
transformation of saikosaponins by gastric juice and intestinal flora. 


33) Hong, T., Jin, GB. and Cyong, JC.: Effect of components of Oren-gedoku-to on murine colitis induced by dextran sulfate sodium. 


*PloS One.*, **4** (9), e7056, 2009.


43) Biriqine, MJ., Eqer, MJ., Williams, HJ., Paulus, HE. and Ward, JR.: TJ-114 (Sairei-To), and herbal medicine in rheumatoid arthritis. 


46) Li, P., Kawachi, H., Morioka, T., Oikawa, M., Oite, T.,

