The Inhibitory Effect of Ergosterol, a Bioactive Constituent of a Traditional Japanese Herbal Medicine Saireito on the Activity of Mucosal-Type Mast Cells

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Mucosal inflammation in ulcerative colitis (UC) is presumed to be regulated primarily by type 2 T helper cell immune responses and mucosal mast cells in the colon are thought to play an important role in the pathogenesis of the mucosal inflammation. Saireito, a Japanese herbal medicine of standardized quality, originating from traditional Chinese medicine (Kampo medicine), is composed of two different Kampo medicines (shosaikoto and goreisan) and is often used for UC in Japan. In this study, we examined the direct effects of these Kampo medicines and their constituents on the antigen-induced degranulation of mucosal-type mast cells. Mucosal-type murine bone marrow-derived mast cells (mBMMCs) were pretreated by these drugs for 24 h, and immunoglobulin E (IgE) receptor-triggered degranulation of mBMMCs was assessed by β-hexosaminidase release. Goreisan showed inhibitory effects on degranulation of mBMMCs in a dose-dependent manner. Among the five constituent medicinal herbs of goreisan, Poria and Polyporus had the inhibitory effects on mBMMCs. Ergosterol, a principal and common component of Poria and Polyporus, also suppressed the degranulation of mBMMCs. Our results provide a molecular basis to explain a portion of the beneficial therapeutic properties of saireito on UC.

Key words: ergosterol; mucosal mast cell; ulcerative colitis; bone marrow-derived mast cell; saireito; goreisan

Ulcerative colitis (UC), an intractable inflammatory disorder of the colon, is characterized by contiguous inflammation of the colonic lamina propria and is thought to be a type 2 T helper cell (Th2) associated disease. Mast cells are pivotal for the colonic immune response and mucosal mast cells in the colon are thought to play an important role in gut hypersusceptibility and inflammation in UC, and we hypothesized saireito has direct effects on mucosal mast cell activation.

In Japan, Japanese herbal medicines of standardized quality, originating from traditional Chinese medicines, so-called Kampo medicines, are widely used for the treatment of various diseases. Among them, saireito, which is often used for the treatment of inflammatory diseases such as rheumatoid arthritis, systemic lupus erythematoses, and nephrotic syndrome, has already been reported to improve the symptoms associated with UC, such as diarrhea and hematochezia; improve the endoscopic findings and quality of life; and reduce the dosage of corticosteroids administered in the combination therapy. However, the precise mechanisms underlying the therapeutic effects of saireito remain largely unclear. Therefore, we examined the effect of saireito on the mucosal oxazolone-induced colitis model resembling human UC, and demonstrated the therapeutic effect of saireito on the UC model. The transcription levels of Th2 cytokines were significantly upregulated in the colon of the colitis mice. In the colon of the saireito-treated mice, the enhanced expression of the Th2 cytokine mRNAs was markedly down-regulated. These results suggest that mucosal mast cells may be greatly involved in the pathogenesis of the inflammatory disease.

The mast cell count is significantly higher in the colonic mucosa of UC patients than in that of patients with Crohn’s disease, Th1 associated inflammatory bowel disease. Mast cells in the colonic mucosa store tryptase in the secretory granules and tryptase secretion is significantly increased in the colonic tissue of UC patients. The result of a pilot study has revealed that systemic administration of APC2059, a specific tryptase inhibitor, is safe and there is evidence of activity in the treatment of UC, suggesting that mucosal mast cells may be greatly involved in the pathogenesis of the inflammatory disease.

Table 1. Medicinal Plant Compositions of Saireito, Shosaikoto and Goreisan

<table>
<thead>
<tr>
<th>Medicinal Plant Compositions of Saireito, Shosaikoto and Goreisan</th>
<th>Saireito (mg/g)</th>
<th>Goreisan (mg/g)</th>
<th>Shosaikoto (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alismatis Rhizoma</td>
<td>12.5</td>
<td>27.6</td>
<td>—</td>
</tr>
<tr>
<td>Poria</td>
<td>7.5</td>
<td>20.7</td>
<td>—</td>
</tr>
<tr>
<td>Polyporus</td>
<td>7.5</td>
<td>20.7</td>
<td>—</td>
</tr>
<tr>
<td>Atractylodis Lanceae Rhizoma</td>
<td>7.5</td>
<td>20.7</td>
<td>—</td>
</tr>
<tr>
<td>Cinnamomi Cortex</td>
<td>5.0</td>
<td>10.3</td>
<td>—</td>
</tr>
<tr>
<td>Bupleuri Radix</td>
<td>17.5</td>
<td>—</td>
<td>29.2</td>
</tr>
<tr>
<td>Pinelliae Tuber</td>
<td>7.5</td>
<td>—</td>
<td>12.5</td>
</tr>
<tr>
<td>Zizyphi Fructus</td>
<td>7.5</td>
<td>—</td>
<td>12.5</td>
</tr>
<tr>
<td>Ginseng Radix</td>
<td>7.5</td>
<td>—</td>
<td>12.5</td>
</tr>
<tr>
<td>Glycyrrhizae Radix</td>
<td>5.0</td>
<td>—</td>
<td>8.3</td>
</tr>
<tr>
<td>Zingiberis Rhizoma</td>
<td>2.5</td>
<td>—</td>
<td>4.2</td>
</tr>
</tbody>
</table>

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Saireito is composed of two kinds of Kampo medicine, shosaikoto and goreisan, and goreisan is composed of five different medicinal plants: *Poria*, *Polyporus*, *Atractylodis Lanceae Rhizoma*, *Alismatis Rhizoma* and *Cinnamomi Cortex* (Table 1). In this study, we examined the effects of saireito, shosaikoto, goreisan and their constituents on IgE-induced degranulation of mucosal-type mast cells.

**MATERIALS AND METHODS**

**Drugs**  Saireito (TJ-114, lot No. 2010114010), shosaikoto (TJ-9, lot No. 2040009010), goreisan (TJ-17, lot No. 2030017010) and the dried powder extracts from constituent medicinal plants were purchased from Tsumura Co. (Tokyo, Japan). These drugs were dissolved in distilled water to the concentration of 10 mg/ml, heated at 95 °C for 20 min, and centrifuged (12000×g for 10 min) to remove insoluble ingredients. The supernatant was filtrated by using 0.45 μm filters for sterilization and used for culturing cells. Ergosterol (Wako Pure Chemical Industries, Osaka, Japan) was dissolved in MeOH at 2.5 mM, then diluted with culture medium, sonicated for 10 min, and used for culturing cells.

**Cell Culture**  We prepared mucosal-type murine bone marrow-derived mast cells (mBMMCs) from a 5- to 7-week-old male mouse (BALB/c) according to the method described previously.10,11 Briefly, bone marrow cells were cultured in RPMI-1640 medium (Sigma, St. Louis, MO, U.S.A.) supplemented with 10% heat-inactivated fetal calf serum (FCS) (JRH Biosciences, Lenexa, KS, U.S.A.), 10 μM 2-mercaptoethanol (Wako Pure Chemical Industries, Osaka, Japan), 20 mM N-(2-hydroxyethyl)piperazine-N’-2-ethanesulfonic acid (Hepes) buffer (Sigma), 1 mM sodium pyruvate (Sigma), 100 μM MEM non-essential amino acids (Sigma), 2 μg/ml gentamicin solution (Sigma), 20 μg/ml penicillin–streptomycin solution stabilized (Sigma), 20 ng/ml recombinant murine interleukin-3 (IL-3; Peprotech, London, U.K.), 40 ng/ml recombinant murine SCF (Peprotech), 5 ng/ml recombinant murine IL-9 (Peprotech) and 1 ng/ml transforming growth factor (TGF)-β1 (Peprotech) at 37 °C in a humidified 5% CO2 atmosphere. Mast cell purity was examined by flow cytometry (FACSCalibur; Becton Dickinson, Franklin Lakes, NJ, U.S.A.), and more than 98% of the non-adherent cells were FcεRI- and c-kit-positive (data not shown). All animal care and experiments were approved by the Animal Experiment Committee in University of Toyama (Authorization No. is INM-13).

**Degranulation Assay**  The degree of degranulation was assessed by measuring β-hexosaminidase release as previously described. Briefly, mBMMCs were pretreated by different concentrations of the test drugs for 24 h, then sensitized with 1.5 μg/ml mouse monoclonal anti-dinitrophenyl (DNP) IgE (Yamasa Co., Tokyo, Japan) for 6 h at 37 °C. The treated cells were resuspended with FACS buffer in polystyrene round-bottom tube. After stained by 2 μg/ml propidium iodide (PI), viability of cells was examined by using FACSCalibur.

**RESULTS AND DISCUSSION**

Saireito and shosaikoto showed significant cytotoxicity to mBMMCs at 1 mg/ml (Fig. 1) and did not show inhibitory effects to cells treated with H2O.

**Fig. 1. Viability of mBMMCs Treated with Saireito, Shosaikoto and Goreisan at 1 mg/ml for 24 h**

mBMMCs were cultured with medium containing the extract of these kampo formulations for 24 h, stained with PI, and analyzed the viability by flow-cytometry. H2O was used as a control. **p < 0.01 compared to cells treated with H2O.**

**Fig. 2. Effect of Goreisan on mBMMC Degranulation**

Goreisan extract at the concentrations noted were added during pre-incubation (24 h) and sensitization (6 h). The treated cells were stimulated with (filled) or without (open) DNP-BSA for 1 h, and β-hexosaminidase release was determined. **p < 0.05 compared to cells treated with H2O.**
effect on the degranulation of mBMMCs at lower concentrations (our unpublished data). In contrast, goreisan did not exhibit the cytotoxicity at 1 mg/ml (Fig. 1). Therefore, we focused on the examination of the direct effect of goreisan on mucosal-type mast cells. Interestingly, the degranulation of mBMMCs was significantly inhibited by treatment with goreisan (H11350 0.32 mg/ml) in a dose-dependent manner (Fig. 2).

Goreisan is composed of five medicinal plants; *Poria*, *Polyporus*, *Atractylodis Lanceae Rhizoma*, *Alismatis Rhizoma* and *Cinnamomi Cortex*. Thus, we investigated which medicinal plant had the inhibitory effect on the degranulation of mucosal mast cells. Among the five medicinal plants, *Poria* (H11350 1 mg/ml), *Polyporus* (H11350 1 mg/ml), and *Cinnamomi Cortex* (H11350 0.32 mg/ml) significantly inhibited the degranulation of mBMMCs (Fig. 3). Neither *Poria* nor *Polyporus* was cytotoxic to mBMMCs at a concentration 1 mg/ml, whereas pretreatment with 1 mg/ml *Cinnamomi Cortex* was cytotoxic (Fig. 4). As assessed by flow cytometry to detect FcεRI and c-kit, the surface expression levels of these receptors were not changed by treatment with *Poria* and *Polyporus* (our unpublished data).

Because the principal and common chemical compound in *Poria* and *Polyporus* is ergosterol,12–14 the effect of ergosterol on the degranulation of mBMMCs was examined. By treating the cells with ergosterol suspension (H11350 32 μM), the degranulation of mBMMCs was significantly inhibited in a dose-dependent manner (Fig. 5). In the present evaluation system, 100 μM ergosterol was not toxic to the mBMMCs (our unpublished data). It has been reported that ergosterol, the principal sterol of most fungi, shows inhibitory activity against 12-O-tetradecanoylphorbol-13-acetate induced ear inflammation in mice.15 In the present study, we demonstrated that ergosterol has the inhibitory effect on the activation of mBMMC. When goreisan was added after the sensitization of mBMMC with anti-DNP IgE, we could not observe the inhibitory effects of goreisan (data not shown), suggesting that goreisam and ergosterol negatively affected the activation process of mBMMC prior to the binding of IgE to FcεRI on mucosal mast cells, which is different from the pharmacological mechanism of mast cell stabilizers.

In conclusion, our results suggest that *Poria*, *Polyporus* and their component ergosterol possess the inhibitory effect on the activity of mucosal-type mast cells. While further studies are needed to fully elucidate the mechanism of these inhibitory effects on mucosal-type mast cells, ergosterol, which is a principal component of these medicinal mushrooms may provide us a lead compound of a therapeutic agent for mucosal mast cell-related diseases.

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**Fig. 3. Effect of Five Constituent Medicinal Herbs of Goreisan on mBMMC Degranulation**

mBMMCs were cultured with medium containing the extracts of *Poria*, *Polyporus*, *Cinnamomi Cortex*, *Atractylodis Lanceae Rhizoma*, and *Alismatis Rhizoma* at the concentrations noted for 24 h and sensitized for 6 h with IgE anti-DNP. Release of β-hexosaminidase was measured as described in Fig. 2. ∗p<0.05 compared to cells treated with H2O.

**Fig. 4. Viability of Cells Treated by *Poria*, *Polyporus* and *Cinnamomi Cortex* at 1 mg/ml for 24 h**

mBMMCs were treated with medium containing the extract of these Kampo medicines for 24 h, and viability of the cells were measured as described in Fig. 1. ∗∗p<0.01 compared to cells treated with H2O.

**Fig. 5. Effect of Ergosterol on the Degranulation of Mast Cells**

Ergosterol was dissolved in MeOH, diluted with medium, and used for culturing cells. mBMMCs were treated with ergosterol at the concentrations for 24 h and release of β-hexosaminidase was measured as described in Fig. 2. Cells treated with or without MeOH were used as controls. ∗p<0.05 compared to cells treated with only MeOH.
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REFERENCES