Effect of components of Oren-gedoku-to (Huang-Lian-Jie-Du-Tang) on murine colitis induced by dextran sulfate sodium

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Abstract

In our previous study, it was found that Oren-gedoku-to (Huang-Lian-Jie-Du-Tang, 黄連解毒湯) (1 g/kg body weight) reduced the histological manifestations of inflammation. In order to investigate which component of Oren-gedoku-to is effective against colitis, we studied the respective action of Coptis Rhizome, Scutellaria Root, Phellodendron Bark, and Gardenia Fruit on DSS murine colitis. The results showed that Scutellaria Root (1 g/kg body weight) restored the lost body weight, increased the hemoglobin content, decreased the gross rectal bleeding and the degree of inflammation, and reduced the histological manifestations of inflammation such as infiltration by polymorphonuclear leukocytes and multiple erosive lesions. Scutellaria Root contained in Oren-gedoku-to, has the strongest pharmacological effect on DSS murine colitis.

Key words colitis, dextran sulfate sodium, inflammatory bowel disease, Coptis Rhizome, Scutellaria Root, Phellodendron Bark, Gardenia Fruit, Oren-gedoku-to.

Abbreviations DSS, Dextran Sulfate Sodium ; PBS, Phosphate Buffered Saline.

Introduction

The pathogenesis of chronic inflammatory bowel disease (IBD) is still unknown. Its etiology is complex and seems to be multifactorial. There is increasing evidence that the immune system plays a critical role in the development and perpetuation of ulcerative colitis (UC) and Crohn's disease (CD).[1-3]

Oren-gedoku-to (Huang-Lian-Jie-Du-Tang, 黄連解毒湯), containing the four components of Coptis Rhizome, Scutellaria Root, Phellodendron Bark and Gardenia Fruit, is a formula of Kampo herbal medicine prescribed ethically in Japan as an effective anti-inflammatory agent. The effect of Oren-gedoku-to on anti-inflammation was also demonstrated in our previous experiment with colitis induced by TNB or DSS.[4] The anti-inflammatory activity of this Kampo herbal medicine has been studied in many aspects, but the precise mechanism is still unclear.[5-7]

As it has been reported that the morphological changes in DSS induced colitis in mice correspond well to the clinical signs of human ulcerative colitis and could serve as a reliable model for studies on its pathogenesis, in order to clarify the components of Oren-gedoku-to which have anti-inflammatory properties, we analyzed the effect of Coptis Rhizome, Scutellaria Root, Phellodendron Bark, and Gardenia Fruit on colitis induced by dextran sulfate sodium.

Materials and Methods

Animals : Specific pathogen-free female BALB/c mice were obtained from Clea Japan, Inc. at 7 weeks of age. The animals were housed in standard cages with wood shavings in a room with carefully

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controlled ambient temperature (25°C) and artificially illuminated (12 hours of light from 8 : 00 AM to 8 : 00 PM), and were fed standard laboratory chow and tap water ad libitum.

Regent : Dextran sulfate sodium (DSS) was purchased from Wako Pure Chemical Industries Inc (Tokyo, Japan).

Administration of dextran sulfate sodium : Mice were divided into 6 groups and given drinking water containing 4 % (wt./vol.) DSS (mol. wt 5000) ad libitum under a regime established for each experiment. The control group was given water only.[8,9]

Morphological analysis : At the end of the experiment, body weight, hemoglobin content and weights of spleen and thymus were measured. To minimize physical artifacts, the removed colon was placed on thick, high quality filter paper without stretching. It was then exposed inside out by cutting longitudinally. Five minutes later, when the tissue fluid in the filter paper had dried, the colonic wall adhered to the filter paper, thus securing a stable fixation. After samples of colonic wall adhering to the filter paper were fixed in 10 % formalin solution (pH 7.2), the medial longitudinal length and weight of each colon were measured.

Grading of histologic changes : Colons were removed on the 10th day and the distal half of the colon was opened longitudinally and embedded in paraffin. Four-μm-thick serial sections were prepared and stained with hematoxylin and eosin for histogic grading. The degree of inflammation in microscopic cross-section of the colon was showed on Table I.

Cytokine assays of splenic lymphocytes : To measure cytokine production, 24-well plates were coated with 10 μg/ml murine anti-CD3e antibody in carbonated buffer (PH 9.6) overnight at 4°C. 2×10^6 splenic lymphocytes were then cultured in 1ml of complete medium in precoated or uncoated well, and 1 μg/ml soluble anti-CD28 antibody was added to the anti-CD3e coated wells. Culture supernatants were removed after 48 hr and assayed for cytokine concentration. IFN-γ and IL-12 concentrations were determined by a specific ELISA kit, according to the manufacturer's recommendation (Bio Source International Inc., Flynn Road, Camarillo, CA, USA). ODs were measured at a wavelength of 490nm.

Treatment with Kampo medicine : Coptis Rhizome (Rhizome of *Coptis chinensis* FRANCH, Sichuan province, China and berberine, 7.22 %), Scutellaria Root (Root of *Scutellaria baicalensis* GEORGI, Hebei province, China and baicalin, 15.88 %), Phellodendron Bark (Bark of *Phellodendron amurense* RUPRECHT, Niigata, Japan and berberine, 4.52 %), and Gardenia Fruit (Fruit of *Gardenia jasminoides* ELLIS, Guangdun province, China) were provided by Uchita Co. Ltd (Tokyo, Japan). The mice were treated daily with 1 g/kg body weight of Coptis Rhizome, Scutellaria Root, Phellodendron Bark, or Gardenia Fruit, respectively from the day when oral DSS water was firstly given.

Statistics : All data were expressed as mean±S.E. The statistical significance of any difference in each parameter among the groups were evaluated by using one-way analysis of variance(ANOVA) followed by Fisher's protected least significant difference(PLSD) comparison tests for Post hoc t-tests. Differences of $P<0.05$ were considered statistically significant, but scores, were analyzed using Wilcoxon's text.

Table I The degree of inflammation in microscopic cross-section of the colon

Score	Ulceration	Epithelium	Infiltration	Lymphoid follicles
0	no ulcers	normal morphology	no infiltrate	no lymphoid follicles
1	one ulcer	loss of goblet cells	infiltrate around crypt bases	1 lymphoid follicles
2	two ulcer	loss of goblet cells in large areas	infiltrate reaching to muscularis mucosae	2 lymphoid follicles
3	3 ulcer	loss of crypts	extensive infiltration reaching the muscularis mucosae ; thickening of the mucosa with abundant oedema	3 lymphoid follicles
4	>3 ulcers	loss of crypts in large areas	infiltration of the submucosa	>3 lymphoid follicles

Results

Effects on general condition of mice with colitis

We found that BALB/c mice subjected to oral administration of 4 % dextran sulfate sodium regularly developed pancolitis with severe diarrhea and rectal prolapse accompanied by extensive wasting disease. In severe cases, gross blood adhering to the anus was noted. From the 3 rd day after administration of DSS, the body weight began to decrease and remained significantly decreased compared with that of normal mice until the 10th day, but administration of Scutellaria Root significantly reversed the loss of body weight. However, the body weight remained increased in the Coptis Rhizome-treated and Phellodendron Bark-treated group, but this was not statistically significant. On the other hand, Gardenia Fruit-treated mice showed significantly lower body weights than those of DSS-treated controls. The results are shown in Figure 1.

The colons of DSS-treated BALB/c mice removed on the 10 th day after oral administration of DSS showed striking hyperemia and inflammation; moreover, the colon and cecum of the DSS mice were significantly shorter than those of the controls, but the colon weight was not significantly different. The severity of ulcerative colitis-like lesions was most marked in the large intestine on the 10th day, when compared with the distal colon of control mice. The administration of Scutellaria Root significantly improved all the symptoms mentioned above. The effects of administration of Coptis Rhizome or Phellodendron Bark were not statistically significant. (Fig. 2)

Effect on hemoglobin content and incidence of occult blood

As shown in Figure 3, the hemoglobin content of the mice treated with 4 % dextran was lower than that of the controls, but the content of those treated with Scutellaria Root was significantly higher than that of the others. The number of mice with occult blood out of 8 mice in each group was 7 in those with DSS colitis, 0 in the control group, 1 in those treated with Scutellaria Root, 2 in those treated with Coptis Rhizome group, 2 in those treated with Phellodendron Bark, 7 in those treated with Gardenia Fruit group.

Effect on damage score and histological changes

Histologically, the distal colon of DSS-treated mice showed inflammatory cell infiltration, with polymorphonuclear leukocytes and multiple erosive lesions, but only in the large intestine. Occasionally,

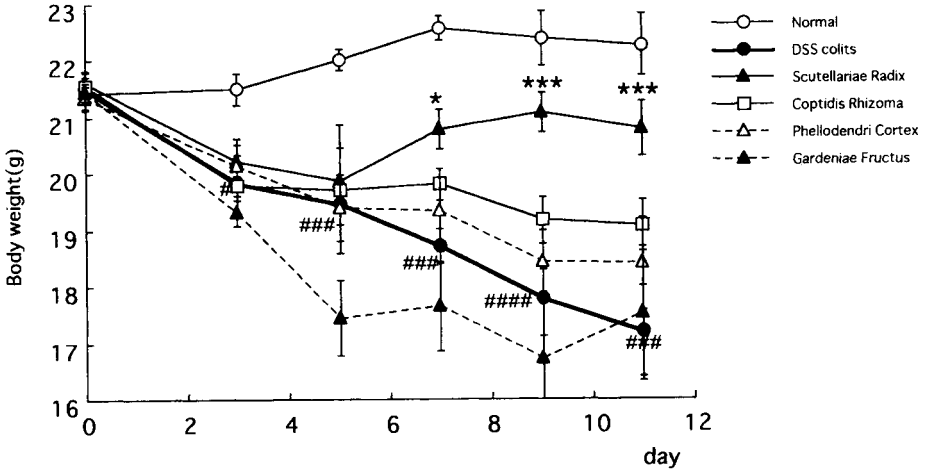

Figure 1 Body weight changes of mice treated with each component of Oren-gedoku-to. Control mice were orally treated with water only, while the DSS model group treated with 4 % DSS in water. Coptis Rhizome, Scutellaria Root, Phellodendron Bark, and Gardenia Fruit (1 g/kg body weight) were given orally from the day of treatment with 4% DSS.*$p<0.05$, ***$p<0.001$ vs DSS model; #$p<0.05$, ###$p<0.001$ vs normal ; Mean±S.E., n=8.

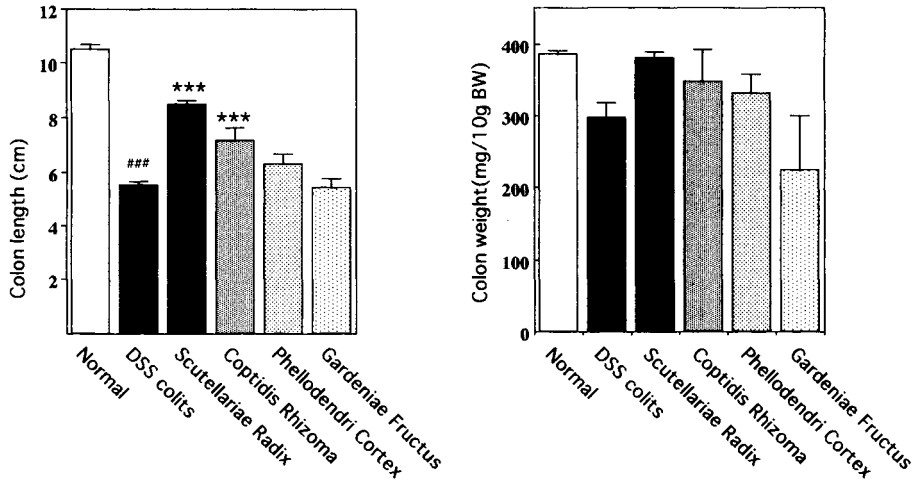

Figure 2 Effects of each component of Oren-gedoku-to on colonic length and weight. The administration of Scutellaria root significantly extended the shortened colonic length, Phellodendron bark or Coptis rhizome extended the shortened colonic length but this effect was not statistically significant; colonic weight was not significantly changed by any component of Oren-gedoku-to. ***$p<0.001$ vs DSS model; ###$p<0.001$ vs normal; Mean±S.E., n=8.

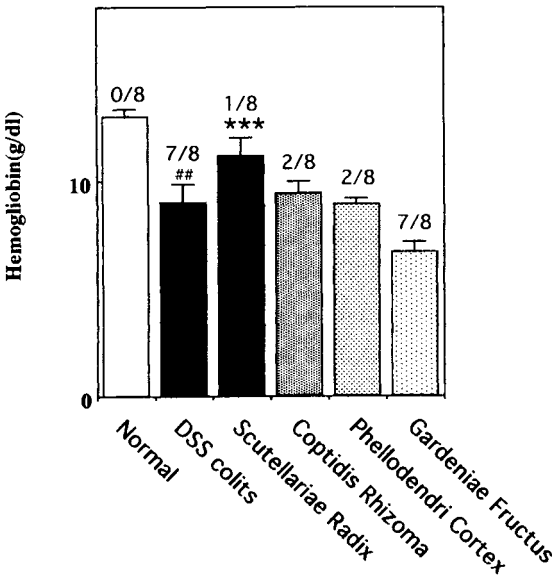

Figure 3 Effects of each component of Oren-gedoku-to on the hemoglobin content and incidence of occult blood .The number of mice with occult blood in the group of 8 DSS mice was 7, that in the control group was 0; but the number in the group treated with Scutellaria root was 1, in those treated with Coptis rhizome group was 2, in those treated with Phellodendron bark was 2, in those treated with Gardenia fruit group was 7. ***$p<0.001$ vs DSS model; ##$p<0.01$ vs normal; Mean±S.E., n=8.

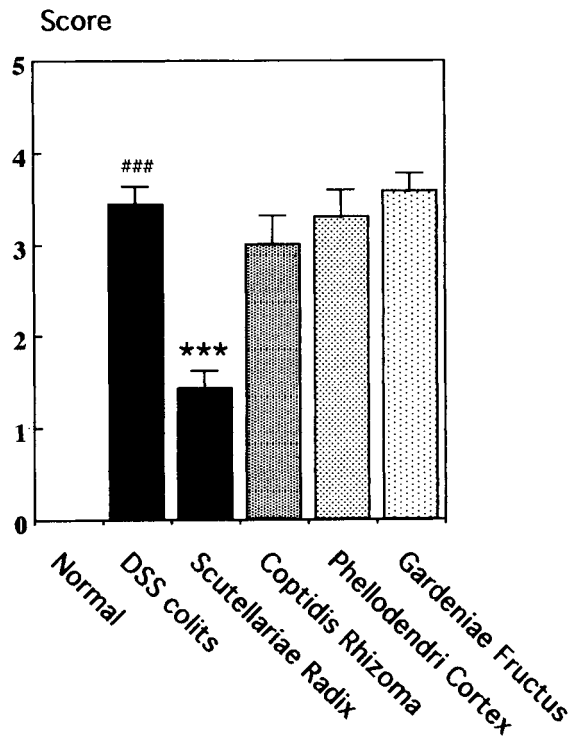

Figure 4 The effect of administration of each component of Oren-gedoku-to on the inflammatory damage score. ***$p<0.001$ vs DSS model; ###$p<0.001$ vs normal; Mean± S.E., n=8.

crypt abscesses and regenerating epithelium were seen in the colonic mucosa. The administration of Scutellaria Root was followed by a significant improvement of these symptoms. (Fig. 4)

Effect on weight of thymus and spleen

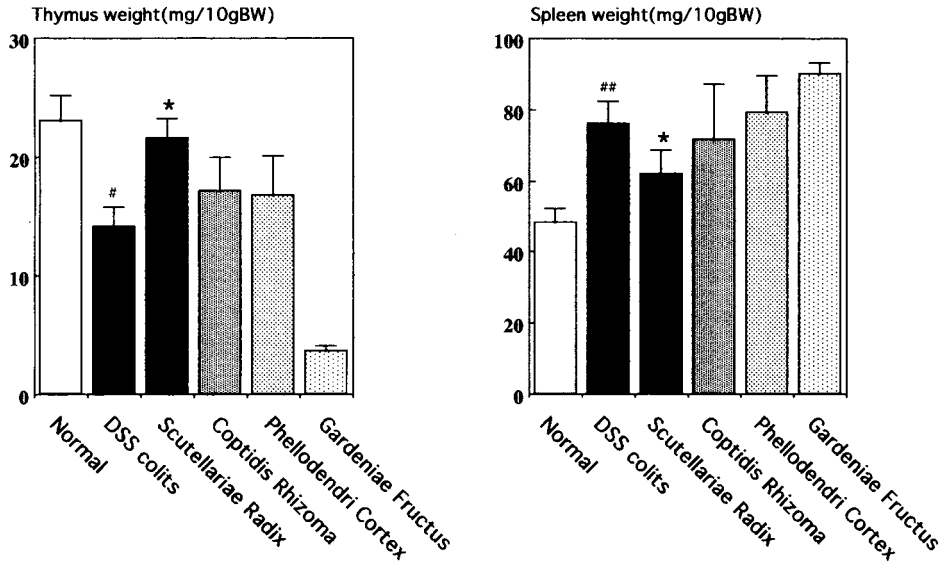

Figure 5 Effect of each component of Oren-gedoku-to on the weight of spleen and thymus in colitis induced by DSS $*p<0.05$ vs DSS model ; $\#p<0.05$, $\#\#p<0.01$ vs normal ; Mean±S.E., n=8.

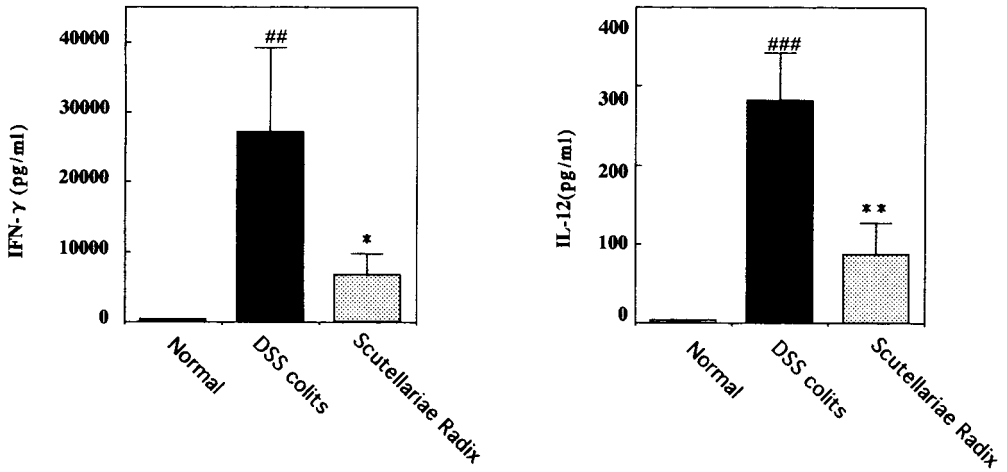

Figure 6 Effect of administration of Scutellaria root on cytokine concentration in splenic lymphocytes. $*p<0.05$, $**p<0.01$ vs DSS model; $\#\#p<0.01$, $\#\#\#p<0.001$ vs normal; Mean±S.E., n=8.

Thymus weights were significantly lower, but spleen weights were significantly higher in mice with DSS colitis than in the controls. The results are shown in Fig. 5. The administration of Scutellaria Root significantly decreased the spleen weight and increased the thymus weight of mice with DSS colitis.

Effects of Scutellaria root on cytokine of splenic lymphocytes

In order to explain the anti-inflammatory mechanism of Scutellaria Root, we analyzed its effects of Scutellaria Root on the IFN-γ and IL-12 content of splenic lymphocytes. The results are shown in Figure 6. IFN-γ and IL-12 were increased in the DSS colitis group. The administration of Scutellaria Root significantly decreased IFN-gamma and IL-12.

Discussion

In the present study, in order to clarify the anti-inflammatory component of Oren-gedoku-to, we analyzed the effect of Coptis Rhizome, Scutellaria Root, Phellodendron Bark and Gardenia Fruit on colitis induced by dextran sulfate sodium. The results showed that Scutellaria Root (1 g/kg body weight) restored the lost body weight, increased the hemoglobin content, decreased the gross rectal bleeding and the degree of inflammation and reduced the histological signs of inflammation such as infiltration by polymorphonuclear leukocytes and multiple erosive lesions. The body weight increased in the Coptis Rhizome-treated and Phellodendron Bark-treated groups, but this was not statistically significant. We demonstrated that Scutellaria Root had the strongest pharmacological effect on DSS murine colitis. Furthermore, we demonstrated that the inflammation was associated with cytokine and could be reversed by Scutellaria Root even after the inflammation was well established.

The cytokine IFN-γ and IL-12 are important molecules involved in inflammation and regulation of the immune response. One of the relevant factors in the initiation, regulation and perpetuation of inflammation in Crohn's disease or ulcerative colitis is a disturbed balance of cytokine. Arguments for these hypotheses are derived from data obtained from mucosal biopsies of patients with IBD: increased expression of proinflammatory cytokines such as IL-1, IL-6, IL-8, IL-12, IFN-γ and TNF-α.[10,11]

Colitis induced by DSS is characterized by ulceration, epithelial damage, mucosal or transmural inflammatory infiltration, and lymphoid hyperplasia. In the present study, our results showed that IFN-gamma and IL-12 concentrations were higher in colitis induced by DSS than in the controls. From the present results, at part of mechanism of Scutellaria Root against DSS-induced colitis may be explained that inhibition of cytokine as IFN-gamma and IL-12 production.

It was reported that baicalein as major compound of Scutellaria root inhibited calcium ionophore A23187-biosynthesis of leukotrienes B4 and C4 in human polymorphonuclear leukocyte.[12] The effect of nine flavonoids isolated from Scutellariae Radix on interleukin-1β and tumor necrosis factor-α-induced adhesion molecule expression in cultured human umbilical vein endothelial cells. Among them, baicalein dose-dependently inhibited IL-1β and TNF-α induced endothelial leukocyte adhesion molecule-1 and intercellular adhesion molecule-1 expressions.[13] Inflammatory bowel disease is characterized by the infiltration of inflammatory cells. Migration of leukocytes into tissues is a central event in the inflammatory response. This migration is mediated by adhesion molecules.[14-16] Therefore, we hypothesize that pharmacological effect of Scutellaria Root may be closely associated with pharmacological effect of baicalein which is contained in Scutellaria Root. So further approach to clarify the effect of Scutellaria Root would be to identify the active components which it contains and to investigate the mechanism of their action on colitis.

In summary, the present data suggest that Scutellaria Root which is contained in Oren-gedoku-to, can prevent colitis induced by DSS and that protective effects may be due to at least in part to regulation of IFN-gamma and IL-12 production.

Acknowledgement

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和文抄録

以前にDSSにより惹起された大腸炎モデルマウスにおいて黄連解毒湯が炎症所見の改善作用および免疫系を正常方向に改善させる作用を示すことを報告した。本研究ではDSS誘発大腸炎モデルに対し黄連解毒湯の構成生薬（黄連，黄柏，黄芩および山梔子）について検討を行った。構成生薬のうち黄連および黄柏にも大腸の病変および免疫系の変化を改善する傾向がみられた。黄芩はDSS誘発大腸炎マウスの体重減少を著明に抑制し，大腸炎の程度も著明に改善した。黄芩はDSS誘発大腸炎マウスの血中ヘモグロビン量の減少を改善した。大腸の出血の有無を判断した結果，黄芩は出血率を著明に抑制し，

大腸の短縮を改善した。黄芩によりDSS誘発大腸炎マウス体重当りの脾臓重量の増加および胸腺重量の低下が有意に抑制された。脾臓のリンパ球のサイトカイン産生について検討した結果T細胞のIFN-gamma及びIL-12産生はDSS大腸炎群で増加していたが，これは黄芩により有意に抑制された。

本研究により，黄連解毒湯の大腸炎抑制作用において，その構成生薬のうち，黄芩が最も重要な役割をもつことが示された。また，その作用機序の一部として，黄芩がサイトカイン産生を抑制することにより，炎症反応を調節していることが示唆された。

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