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

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Abstract

In the present study, experimental ulcerative colitis models were produced in mice by providing them with drinking water containing synthetic dextran sulfate sodium. Mice that developed acute colitis showed signs of diarrhea, gross rectal bleeding and weight loss within 10 days. It was found that Oren-gedoku-to (1 g/kg body weight) restored the body weight which was lost, increased the hemoglobin content and decreased the gross rectal bleeding. We demonstrated that Oren-gedoku-to not only decreased the degree of inflammation, but also improved the histological signs of inflammation such as infiltration by polymorphonuclear leukocytes and multiple erosive lesions. Furthermore, analysis of splenic lymphocytes showed that Oren-gedoku-to increased the LFA-1 expression on $CD3^+$ cell and decreased the LFA-1 expression on other cells. We demonstrated that successful treatment of established DSS colitis can be achieved by administration of Oren-gedoku-to.

Key words colitis, CD11a/LFA-1, CD3, dextran sulfate sodium, inflammatory bowel disease, Oren-gedoku-to (Huang-Lian-Jie-Du-Tang, 黄連解毒湯).

Introduction

Inflammatory bowel disease (IBD) is comprised of two forms of chronic intestinal inflammation: Crohn's disease and ulcerative colitis. While the precise etiology of each of these forms of inflammation remains unknown, it is increasingly clear that the mechanism of colitis is related to a disordered immune system.[1-3] In approaches to the understanding of the immunopathogenesis of BID, various investigators have focused on murine models of mucosal inflammation resembling BID. These models include 2,4,6-trinitrobenzenesulfonic acid colitis, dextran sulfate sodium colitis and others.

Oren-gedoku-to (Huang-Lian-Jie-Du-Tang), containing four components of Coptis rhizome, Scutellaria root, Phellodendron bark, and Gardenia fruit, is a formula of Kampo herbal medicine applied ethically in Japan as an effective anti-inflammatory agent. Oren-gedoku-to has been used for a long time in the treatment of various diseases, such as gastritis, dermatitis, aphthous stomatitis and hypertension. In animal experiments, it has been used to determine its haemostatic effect, anti-hypertensive action and inhibitory effect on gastric mucosal lesions.[4] It was recently reported that Oren-gedoku-to possessed an anti-inflammatory action in experimental colitis induced by 2,4,6-trinitrobenzene sulfonic acid and that it inhibited IL-8 and SOD in the serum of rats subjected to acetic acid-induced inflammation.[5,6] Anti-inflammatory activity was studied in many aspects of the investigation, but the precise mechanism was still unclear.

The effect of Oren-gedoku-to on anti-inflammatory activity was also demonstrated in our previous experiment of colitis induced by TNB. It was reported that the lamina propria T cell responses induced by TNB colitis showed a similarity to those observed in human Crohn's disease. It was also reported that the

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morphological changes in DSS induced colitis in mice correspond well to the clinical signs of human ulcerative colitis and can serve as a reliable model for studies on its pathogenesis. Melatonin and LK 423, a new phthalimido compound were effective on DSS-induced colitis.[7,8] In this study, in order to clarify these anti-inflammation mechanisms and to determine whether it is possible improve the immune function in colitis, we analyzed the effect of Oren-gedoku-to 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 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 3 groups and given drinking water containing 4 % (wt./vol.) DSS (mol. wt 5000) [9] ad libitum under a regime established for each experiment. In control experiments, mice received water only.

Morphological analysis : All analyses were performed 'blind'. After the experiment, body weight, hemoglobin content and weights of spleen and thymus were measured. To minimize physical artifacts, the removed colon was put 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. 4-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 graded as follows : ulceration : 0, no ulcers; 1, one ulcer ; 2, two ulcers ; 3, three ulcers; 4, >3 ulcers; epithelium : 0, normal morphology ; 1, loss of goblet cells ; 2, loss of goblet cells in large areas ; 3, loss of crypts ; 4, loss of crypts in large areas; infiltration : 0, no infiltrate ; 2 infiltration around crypt bases ; 2, infiltration reaching to muscularis mucosae ; 3, extensive infiltration reaching the muscularis mucosae ; thickening of the mucosa with abundant oedema ; 4, infiltration of the submucosa; lymphoid follicles : 0, no lymphoid follicles ; 1, one lymphoid follicles ; 2, two lymphoid follicles ; 3, three lymphoid follicles ; 4, >3 lymphoid follicles.

Cell isolation and purification of splenic lymphocytes : After BALB/c mice were killed, the spleen was immediately removed and pressed with a slide glass in phosphate-buffered saline. The cell suspension was centrifuged at 1500 rpm for 5 min at 4 °C temperature, and the suspension was collected and washed 3 times with PBS (-) after 5 ml 0.8 % NH_4Cl solution had been added.

Antibodies : Monoclonal antibodies used for flow cytometric analysis were phycoerythin (PE)-conjugated rat anti-mouse CD3IgG and fluorescein isothiocyanate (FITC)-conjugated rat anti mouse CD11a IgG.

Flow cytometric analysis : Spleen lymphocytes were incubated with 1 μg/million cells of fluorescein-conjugated antibodies for 1hr at 4°C. Fluorescence-activated cell were washed 3 times with PBS (-) and analyzed by EPICS XL flow cytometry (Coulter Cytometry Co., Hialeah, FL, USA). A fluorescence histogram of at least 5,000 counts was collected for each sample.

Treatment with Kampo medicine : Kampo medicine, Oren-gedoku-to (OGT), was provided by Tsumura Co. (Tokyo, Japan). The mice were treated with 1 g/kg body weight of Oren-gedoku-to daily from the day when oral DSS water was first 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 signifiant difference(PLSD) comparison tests for *Post hoc* t-tests. Differences of $P<0.05$ were considered statistically significant, except for scores, which were analyzed using Wilcoxon's rank sum test.

Results

Effects on general condition of mice with colitis

We found that BALB/c mice subjected to oral administration of 4 % dextran sulfate sodium reproducibly developed pancolitis with severe diarrhea and rectal prolapse accompanied by an extensive wasting disease. In severe cases, gross blood adhering to the anus was noted. Administration of Oren-gedoku-to significantly reversed the loss of body weight in DSS-induced colitis. The result is shown in Figure 1.

From the 4 th day after administration of DSS, the body weight began to decrease and significantly decreased compared with normal mice until the 10 th day, but treatment with Oren-gedoku-to resulted in an improvement of the general condition of the mice.

The colons of DSS-treated BALB/c mice removed on the 10 th day after oral administration of DSS revealed 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 changed. The

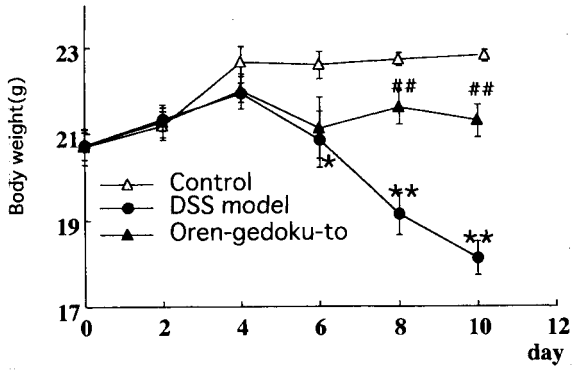

Figure 1 Body weight changes of mice treated with Oren-gedoku-to. Control mice were orally treated with distilled water, DSS model group treated with 4 % DSS water. Oren-gedoku-to group(1 g/kg body weight) were orally treated from the day of treatment with 4 % DSS water. $*p<0.05$ vs control; $**p<0.001$ vs control; $\#\#p<0.001$ vs DSS model; Mean±S.E., n=8.

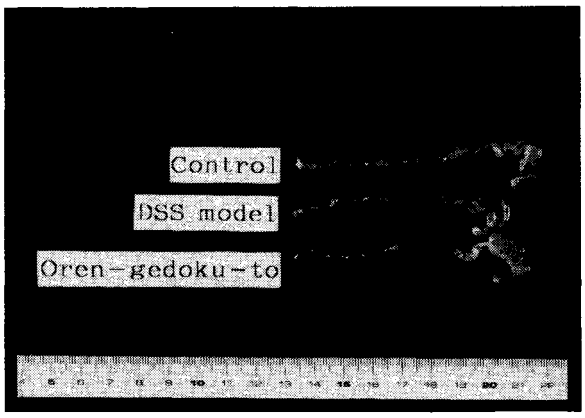

Figure 2 Macroscopic changes of colon and spleen in DSS-treated mice. Photographs of large intestine of BALB/c mouse. The mice were treated with distilled water (top row) and treated with 4 % DSS (second row), and those treated with DSS were given Oren-gedoku-to(1 g/kg) (bottom row). The colons of the DSS-treated mice are severely inflamed and hyperemic, and they contain less feces due to massive diarrhea, but the inflammation and heperemic of DSS murine colitis was improved by administration of Oren-gedoku-to.

severity of ulcerative colitis-like lesions was most marked on the left side of the large intestine on the 10 th day, when compared with distal colon of control mice. The administration of Oren-gedoku-to significantly improved all the above-mentioned symptoms.

Effect on hemoglobin content and incidence of occult blood

As shown in Figure 4, the hemoglobin content of the mice treated with 4 % dextran was lower than that of the controls, but the hemoglobin content of those treated with Oren-gedoku-to was 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 and 1 in those treated with Oren-gedoku-to group.

Effect on damage score and histologic changes

Histologically, the distal colon of DSS-treated mice showed inflammatory cell infiltration, including polymorphonuclear leukocytes and multiple erosive lesions, but only in the large intestine. Occasionally, crypt abscesses and regenerating epithelium were seen in the colonic mucosa. In these mice, the severity of ulcerative colitis-like lesions was more marked on the left side of the large intestine on the 10 th day, when compared with distal colon from control mice. The administration of Oren-gedoku-to resulted in a

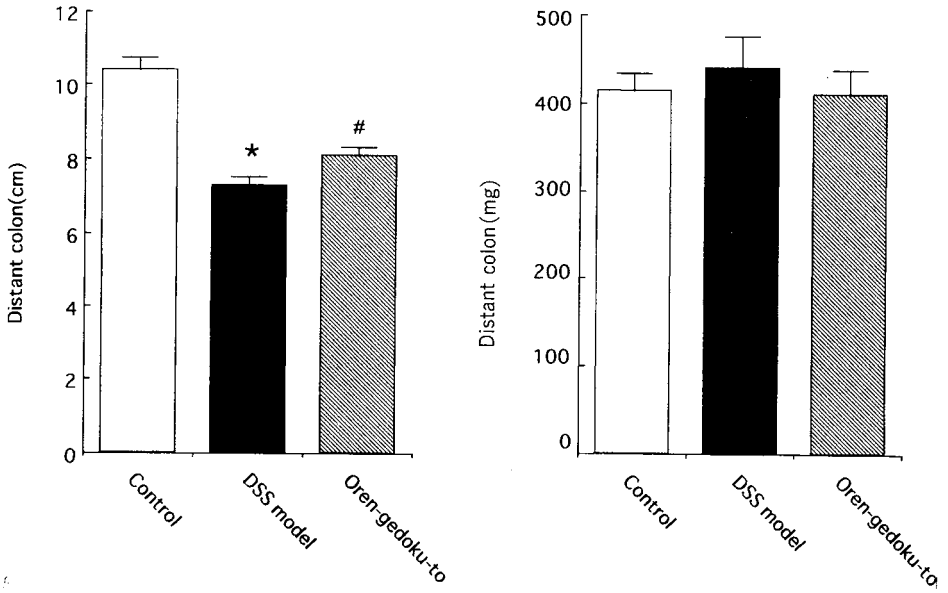

Figure 3 Effects of Oren-gedoku-to on colonic length and weight: the administration of Oren-gedoku-to significantly extended the shortened colonic length, but colonic weight was not significantly changed. *$p<0.05$ vs control; #$p<0.05$ vs DSS model; Mean±S.E., n=8.

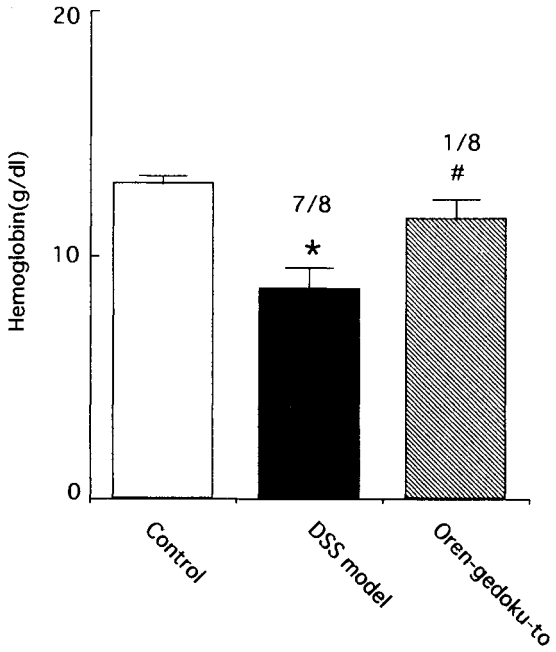

Figure 4 Effects of Oren-gedoku-to on the hemoglobin content and incidence of occult blood in mice. The number of mice with occult blood out of 8 mice in each group was 7 in those with DSS mice, 0 in the control group, 1 in those treated with Oren-gedoku-to group. *$p<0.05$ vs control; #$p<0.05$ vs DSS model; Mean±S.E., n=8.

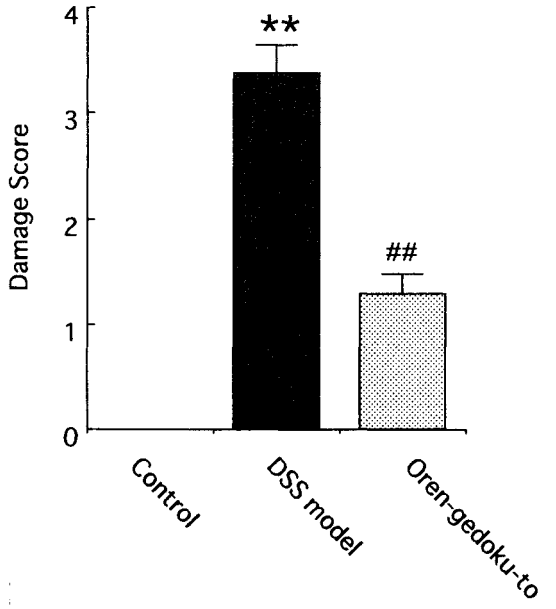

Figure 5 The effect of administration of Oren-gedoku-to on the damage score of inflammation. **$p<0.001$ vs control; ##$p<0.001$ vs DSS model; Mean±S.E., n=8.

significant improvement of these symptoms.

Effect on weight of thymus and spleen

Thymus weights were significantly lower, but spleen weights were significantly higher in mice suffering from DSS colitis than in the controls. The

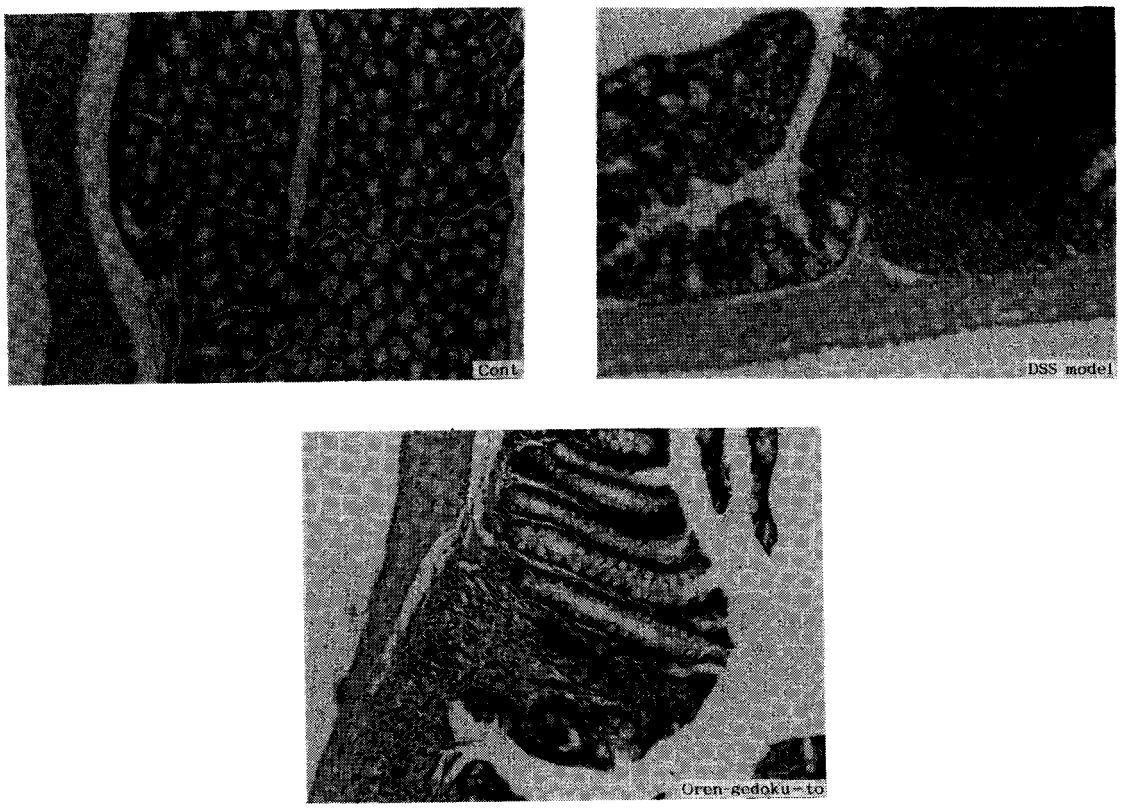

Figure 6 Typical histological appearance of the colon. Cont : normal ; DSS model : DSS-treated mice; Oren-gedoku-to : DSS-treated mice+Oren-gedoku-to (1 g/kg). Original magnification x 100.

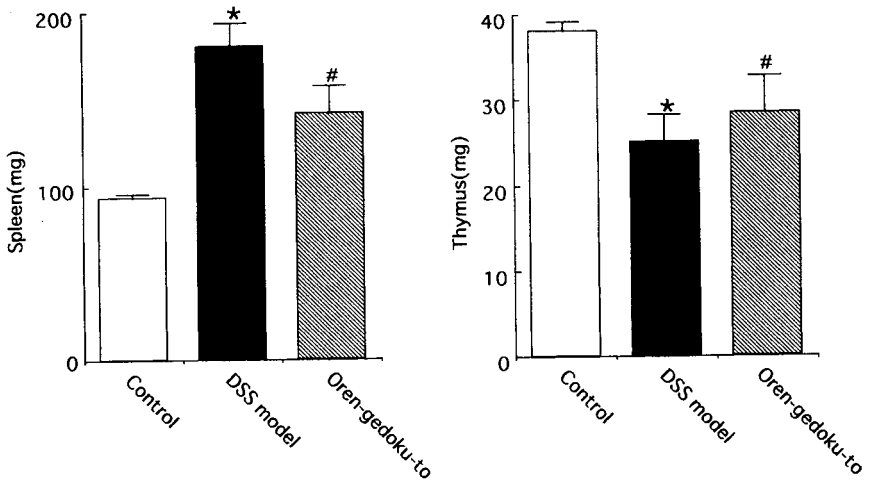

Figure 7 Effect of Oren-gedoku-to on the weight of spleen and thymus in colitis induced by DSS.
* : Significantly different from the control at $p < 0.05$.
# : Significantly different from the DSS colitis model at $p < 0.05$.

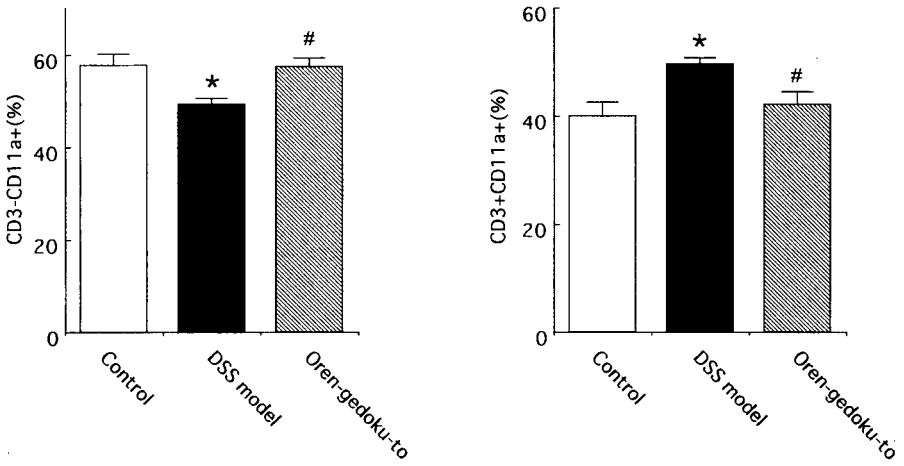

Figure 8 Effect of administration of Oren-gedoku-to on the LFA-1 expression of splenic lymphocytes. $*p<0.05$ vs control ; $\#p<0.05$ vs DSS model ; Mean±S.E., n=8.

results are shown in Fig. 7.

The administration of Oren-gedoku-to significantly decreased the spleen weight and increased the thymus weight of those with DSS colitis.

Effects on LFA-1 expression of splenic lymphocytes

The results are shown in Figure 8. The LFA-1 expression of $CD3^+$ lymphocytes was increased and that of other lymphocytes was decreased in DSS colitis. The administration of Oren-gedoku-to decreased LFA-1 expression of $CD3^+$ lymphocytes and increased LFA-1 expression of other splenic lymphocytes.

Discussion

In the present study, we described treatment with Oren-gedoku-to of a novel murine model of intestinal inflammation induced by administration of dextran sulfate sodium. It was found that Oren-gedoku-to reversed the loss of body weight induced by DSS, but it also decreased the inflammation score. Furthermore, we demonstrated that Oren-gedoku-to significantly increased the weight of the thymus and decreased the weight of the spleen affected by DSS, and that the inflammation associated with expression of LFA-1 and could be reversed by Oren-gedoku-to even after inflammation was well established.

In addition to previous ulcerative model using guinea pigs, hamsters and rabbits, a novel method of inducing ulcerative colitis in mice by DSS administration was recently developed. This experimental rodent model of ulcerative colitis showed clinical symptoms including diarrhea, occult blood, and gross rectal bleeding. In presenting this colitis model, shortening of the colon was also observed after administration of DSS-distilled water. Further, possibly in relation to the inflammatory changes, lymphoid follicles were frequently found in the colonic wall beside prominent regenerative changes in the colonic mucosa ; moreover, high-grade dysplasia was found. The changes could be attributed to the recurrence of inflammatory ulceration and regeneration of colonic mucosa. Inflammatory changes in colitis induced by carrageenan or sulfate amylopectin are prominent in the cecum, a feature that differs from the results seen in DSS-induced colitis. This appears to be acute, while carrageenan induced colitis occurred only after long-term administration. The morphological changes in DSS induced colitis correspond well to the clinical signs of human ulcerative colitis, and mice can thus serve as a reliable model for studies on its pathogenesis. In the present study, we demonstrated that Oren-gedoku-to significantly improved the above-mentioned histological change induced by DSS.

The β_2 integrins are heterodimers of α subunits linked with the CD18β subunits. This family of integrins is expressed on lymphocytes and includes lymphocyte function-associated antigen-1 (LFA-1)

(CD11a/CD18), CR3/mac-1 (CD11b/CD18) and P150.95 (CD11c/CD18). The LFA-1/ICAM pathway, in particular, is likely to be pivotal in the immunopathogenesis of inflammatory bowel disease, because: a) it mediates leukocyte adhesion and transmigration across the endothelium, b) it is important in the costimulation of mucosal T cells, and c) the expression of these surface molecules is intimately associated with a variety of inflammatory cytokines, known to be increased in the intestinal milieu in IBD. Increased expression of adhesion molecules has been noted in numerous diseases that have an inflammatory component. The migration of leukocytes and lymphocytes into tissues is the central event in inflammation and this is an immune response. In inflammatory bowel disease, there is a dense intestinal infiltrate of inflammatory and activated immune cells with a different distribution pattern for Crohn's disease and ulcerative colitis. For the development of the circulating local intestinal cellular infiltrate, circulating cells must adhere and transmigrate so that a reaction is created.[10,11] In our study, LFA-1 expression in DSS colitis was increased on T lymphocytes, but decreased on other lymphocytes. On treatment with Oren-gedoku-to, these changes in LFA-1 expression were reversed. The further aim of our approach is to clarify the effect of Oren-gedoku-to and to investigate the production of cytokine in the local mucosa and general immune system.

和文抄録

黄連解毒湯はDSS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抑制した。体重当りの脾臓重量は有意に減少したが，胸腺の重量は，黄連解毒湯により有意に増加した。黄連解毒湯はDSS大腸炎マウスの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，黄連解毒湯は出血率を著明に抑制し，大腸長さも増加した。T 細胞のCD11aの発現率はDSS大腸炎群で増加していた。黄連解毒湯はDSS大腸炎のCD11a発現率増加を抑制した。DSSにより惹起された大腸炎モデルマウスにおいても黄連解毒湯は炎症所見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した。

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