RESEARCH ARTICLE

Xanthoangelol Isolated from Angelica keiskei Roots Prevents Dextran Sulfate Sodium-Treated Colitis in Mice

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Abstract: Background: The therapeutic effects of a number of natural products on Inflammatory Bowel Disease (IBD) have recently been examined in detail. The whole herb and roots of Angelica keiskei (Umbelliferae) have traditionally been used as a diuretic, to treat gastrointestinal diseases such as gastric ulcers and diarrhea in Japan. Objectives: The present study was performed to investigate the effects of xanthoangelol, a major chalcone of Angelica keiskei roots, on diarrhea and inflammation in the large intestine of IBD model mice. Methods: Xanthoangelol (10 & 25 mg/kg) was orally administered to mice with 3% Dextran Sulfate Sodium (DSS)-induced colitis. Blood samples were collected during the experimental period, subjected to a full blood count test, and colonic cytokine and chemokine levels were measured. Results: Xanthoangelol (25 mg/kg) reduced the Disease Activity Index (DAI) of colitis. It also attenuated DSS-induced reductions in red blood cell and platelet counts as well as Hb and Ht levels. A histological examination of the colon using direct fast scarlet staining showed that xanthoangelol prevented DSS-induced mucosal ulceration and eosinophil infiltration. Xanthoangelol also reduced DSS-induced increases in colonic MCP-1, IL-1β, and TNF-α levels. Conclusion: Xanthoangelol reduced DSS-induced increases in colonic IL-1β, TNF-α, and MCP-1 levels and prevented eosinophil infiltration, which supports its potential as a treatment for IBD.

Keywords: Xanthoangelol, dextran sulfate sodium, inflammatory bowel disease, interleukin 1β, tumor necrosis factor-α, monocyte chemoattractant protein 1, eosinophil infiltration.

1. INTRODUCTION

The grass and roots of Angelica keiskei Koizumi (Umbelliferae) have traditionally been used as a diuretic, to treat gastrointestinal diseases (including nausea, gastraparesis, gastric atony, and diarrhea), and as a tonic in Hachijyou island, Japan. Previous studies reported that the extract and xanthoangelol (major chalcones) of this plant exhibited anti-platelet platelet [1], the inhibition of Helicobacter pylori-induced gastric inflammation [2], anti-oxidative and anti-hyperlipidemic activities [3], anti-thrombotic [4], the prevention of obesity-induced plasminogen activator inhibitor (PAI)-1 elevation [5], and the inhibition of dexamethasone-induced muscle atrophy by stimulating myoblast differentiation [6]. Diseases of the large intestine, including Inflammatory Bowel Disease (IBD), have recently been the focus of many studies. IBD is a general term for chronic gastrointestinal inflammatory diseases and two main types are Crohn’s Disease (CD) and Ulcerative Colitis (UC). The number of Japanese patients with IBD (Designated intractable disease no. 97) was higher than 160,000 in 2013 and has continued to increase [7]. The standard treatments for patients with IBD, including CD and UC, are corticosteroids, 5-aminosalicylic acid, immunomodulators, such as thiopurine and methotrexate [8, 9], biological drugs, and anti-Tumor Necrosis Factor (TNF) antibodies [10]. We previously demonstrated the artery relaxation [11] as well as anti-tumor and antimetastatic [12-14] effects of xanthoangelol. The present study was performed to examine the effects of xanthoangelol (E)-1-[3-{(2E)-3,7-dimethylocta-2,6-dieyl}-2,4-dihydroxyphenyl]-3-(4-hydroxyphenyl)prop-2-en-1-one] (Fig. 1) on Dextran Sulfate Sodium (DSS)-induced colitis in mice.

2. MATERIALS AND METHODS

2.1. Materials

Xanthoangelol [15-17] (Fig. 1) was supplied by Honorary Professor K. Baba (Department of Pharmacognosy, Osaka University of Pharmaceutical Sciences, Osaka, Japan).
DSS (molecular weight, 36,000 to 50,000) was purchased from MP Biochemicals (CA, USA), 10% buffered formalin solution from Fuji-Wako Pure Chemical Co. (Osaka, Japan), and 0.9% NaCl solution (saline) from Otsuka Pharmacy Co. (Tokushima, Japan). The anesthetics medetomidine hydrochloride (Domitor®), midazolam (Dormicum®) and butorphanol tartrate (Vetorphale®) were obtained from Nippon Zenyaku Kogyo Co., Ltd. (Fukushima, Japan), Astellas Pharm Inc. (Tokyo, Japan), and Meiji Seika Pharma Co. (Tokyo, Japan), respectively.

![Figure 1](image-url) Structure of xanthoangelol.

2.2. Animals

Six-week-old male C57BL/6J mice were purchased from Japan SLC Co. (Shizuoka, Japan). They were housed for seven days in a temperature-controlled room at 25 ± 1°C and 60% relative humidity, and fed standard laboratory diet (Oriental Yeast Co., Osaka, Japan) and water ad libitum prior to the initiation of experiments. All experiments conformed to the Ethical Guidelines for Animal Experimentation, Ehime University, the Japanese Pharmacological Society, and the Guide for the Care and Use of Laboratory Animals by the National Institute of Health, and were approved by the Ethics Committee on Animal Experimentation, Ehime University (approval no. YA-1-1).

2.3. Preparation of Anesthesia

Medetomidine hydrochloride (0.75 ml), midazolam (2 ml), and butorphanol tartrate (2.5 ml) were mixed and the final volume for injection was adjusted to 20 ml by distilled water. The intraperitoneal administration of anesthetic was performed at 0.1 ml per 10 g body weight.

2.4. Effects of Xanthoangelol on DSS-Induced Colitis

Mice were divided into the following groups of six mice each: a normal group, DSS-treated group, and xanthoangelol (10 and 25 mg/kg twice daily) DSS-treated groups. To induce experimental colitis, mice were orally given in the drinking water ad libitum containing 3% (w/v) DSS solution for 7 days. Xanthoangelol (10 and 25 mg/kg) was orally administered to DSS-treated mice twice daily (7:00 and 17:00) for one week. Body weight, the intakes of food and DSS, and drinking water reduce experimental colitis, mice were orally given in the (10 and 25 mg/kg twice daily) DSS-treated groups. To administer to DSS-treated mice twice daily (7:00 and 17:00) for 7 days. Xanthoangelol (10 and 25 mg/kg) was orally administered to DSS-treated mice twice daily (7:00 and 17:00) for 7 days.

2.5. Measurement of the Large Intestine, Spleen, and Thymus Weights, Red Blood Cell, Leukocyte, and Platelet Counts, and Hemoglobin (Hb) and Hematocrit (Ht) Levels in DSS-treated Mice

Blood samples were collected on day 8 by venipuncture under anesthesia, mice were euthanized by cervical dislocation, and the large intestine, spleen, and thymus were then removed. The length and weight of the large intestine and the weights of the spleen and thymus were measured to assess the DAI of colitis and any side effects. Blood samples were collected in heparin-coated tubes, and red blood cell, leukocyte, and platelet counts as well as Hb and Ht levels were analyzed with a Coulter Counter (Japan Scientific Instruments Co., Ltd., Tokyo, Japan).

Table 1. Disease activity index.

<table>
<thead>
<tr>
<th>Weight Loss Score</th>
<th>Stool Score</th>
<th>Bleeding Stool Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>0: None</td>
<td>0: Normal</td>
<td>0: Negative</td>
</tr>
<tr>
<td>1: 1 - &lt; 5%</td>
<td>1: Light loose</td>
<td>1: Light surface positive</td>
</tr>
<tr>
<td>2: 5 - &lt; 10%</td>
<td>2: Loose</td>
<td>2: Hemoccult positive</td>
</tr>
<tr>
<td>3: 10 - &lt; 15%</td>
<td>3: Light diarrhea</td>
<td>3: Bleeding</td>
</tr>
<tr>
<td>4: &gt;15%</td>
<td>4: Severe diarrhea</td>
<td>4: Gross bleeding</td>
</tr>
</tbody>
</table>

2.6. Measurement of Tumor Necrosis Factor-α (TNF-α), Interleukin 1β (IL-1β), and Chemokine Monocyte Chemoattractant Protein 1 (MCP-1) Levels in the Blood and Large Intestine of DSS-Treated Mice

After washing in Phosphate-Buffered Saline (PBS, pH 7.0), the large intestine (approximately 100 mg) was cut into small pieces. Tissue Protein Extraction Reagent (T-PER, Pierce Co., Rockford, IL, USA) containing protease inhibitor (Fuji-Wako Pure Chemical Co., Tokyo, Japan) (2 ml) was added and the mixture was homogenized, followed by centrifugation at 2,000×g at 4 °C for 15 min. TNF-α, IL-1β and MCP-1 levels in the supernatant were assessed using the respective Enzyme-Linked Immunosorbent Assay (ELISA) kits (R & D Systems, Minneapolis, MN, USA). Blood samples were subjected to the same assays.

2.7. Histological Analysis of Mucosal Ulceration and Eosinophil Infiltration in DSS-Treated Mice

After gentle washing in ice-cold PBS (pH 7.0), the large intestine was fixed in 10% buffered formalin for at least 24 h, dehydrated in solutions containing increasing percentages of ethanol (70, 80, 95 and 100%, v/v), and cleared in Histo-clear (AS-One, Tokyo, Japan). Tissues samples were then embedded in paraffin under a vacuum, sectioned at a thickness of 5 μm, deparaffinized, and stained with direct fast scarlet (DFS) (to detect mucosal ulceration and eosinophil infiltration) and Hematoxylin-Eosin (HE) (to detect mucosal ulceration). Saga [19] recently demonstrated the staining of eosinophils in tissues by DFS. The same cross-sections were selected from three plates per sample, and images were taken from four different microscopic fields (40×, 100×, 200×, or 400×) per plate. Mucosal thickness was measured using a Digimatic Caliper (Mitsumoto Co., Kanagawa, Japan) and the number of DFS-positive eosinophils was counted.

2.8. Statistical Analysis

Data were shown as means ± SEM and assessed by a one-way or repeated-measure analysis of variance (ANOVA). When the F-test was significant, means were compared by Dunnett’s test with Stat View (SAS Institute Co., Tokyo,
Xanthoangelol Prevents DSS-Induced Colitis

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3. RESULTS

3.1. Body Weight, DAI, and Spleen and Thymus Weights in DSS-Treated Mice

Body weights from Days 5 to 7 were significantly lower in DSS-treated mice than in control mice (Fig. 2a). Xanthoangelol (25 mg/kg) significantly inhibited DSS-induced reductions in body weight on Day 7 (Fig. 2a). DSS reduced food intake by mice. Xanthoangelol (25 mg/kg) attenuated DSS-induced decreases in food intake on Days 6-7 (Fig. 2b). Xanthoangelol (25 mg/kg) inhibited the aggravations of stool conditions and bleeding stool on Days 3-5 compared to those of DSS-treated groups (control) (Figs. 2c and 2d). The administration of xanthoangelol (25 mg/kg) also reduced DAI (Fig. 2e) on Days 3-5.

Spleen weights were not significantly different between the control, DSS-treated, and DSS-treated xanthoangelol-administered (10 and 25 mg/kg) groups (Table 2). However, thymus weights were lower in DSS-treated mice than in control mice. Xanthoangelol (25 mg/kg) significantly attenuated DSS-induced decreases in thymus weights (Table 2).

3.2. Red Blood Cell, Leukocyte, and Platelet Counts as well as Hb and Ht Levels in DSS-Treated Mice

Red blood cell and platelet counts, as well as Hb and Ht levels, were significantly lower in DSS-treated mice than in control mice (Table 3). Xanthoangelol (10 and 25 mg/kg) inhibited these DSS-induced reductions.

3.3. Colon Length and Histology in DSS-Treated Mice

On day 8, colon lengths in the normal, DSS-treated (control), and DSS-treated plus xanthoangelol-administered (10 and 25 mg/kg) groups are shown in Fig. (3) at the final day.
Table 2. Effects of xanthoangelol on spleen and thymus weights in dextran sulfate sodium-treated C57Bl J mice.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Spleen (mg)</th>
<th>Thymus (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>88.20 ± 4.71</td>
<td>47.48 ± 5.83*</td>
</tr>
<tr>
<td>3% DSS (Control)</td>
<td>103.85 ± 12.40</td>
<td>18.90 ± 2.17</td>
</tr>
<tr>
<td>+ Xanthoangelol (10 mg/kg)</td>
<td>80.25 ± 14.52</td>
<td>20.28 ± 3.49</td>
</tr>
<tr>
<td>+ Xanthoangelol (25 mg/kg)</td>
<td>92.20 ± 7.30</td>
<td>31.65 ± 3.38*</td>
</tr>
</tbody>
</table>

Values are the means ± SE of 6 mice.
*Significantly different from dextran sulfate sodium-treated groups; *P<0.05.

Table 3. Effects of xanthoangelol on red blood cell, leukocyte, and platelet counts as well as hemoglobin (Hb) and hematocrit (Ht) levels in dextran sulfate sodium-treated mice.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Red cells (*10^6/mL)</th>
<th>Leukocytes (*10^3/mL)</th>
<th>Platelets (*10^4/mL)</th>
<th>Hb (g/100 mL)</th>
<th>Ht (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>807.0±25.27*</td>
<td>1.12±0.36</td>
<td>98.78±5.96*</td>
<td>12.52±0.40*</td>
<td>37.47±1.15*</td>
</tr>
<tr>
<td>3% DSS (Control)</td>
<td>302.67±44.09</td>
<td>2.05±0.52</td>
<td>15.77±2.58</td>
<td>4.90±0.67</td>
<td>13.67±1.92</td>
</tr>
<tr>
<td>+ Xanthoangelol (10 mg/kg)</td>
<td>470.80±31.12*</td>
<td>0.92±0.21*</td>
<td>45.88±4.77*</td>
<td>7.54±0.47*</td>
<td>21.10±1.41*</td>
</tr>
<tr>
<td>+ Xanthoangelol (25 mg/kg)</td>
<td>612.00±53.45*</td>
<td>0.83±0.20*</td>
<td>88.13±10.43*</td>
<td>9.52±0.83*</td>
<td>27.63±2.34*</td>
</tr>
</tbody>
</table>

Values are the means ± SE of 6 mice.
*Significantly different from dextran sulfate sodium-treated groups; P<0.05.

Fig. (3). Effects of xanthoangelol on colon length (a and b) in 3% DSS-treated mice. Values are the means ± SEM of six mice. * Significantly different from 3% DSS-treated mice. P<0.05. 3% DSS: 3% dextran sulfate sodium.
Fig. (4). Light micrographs of DFS (a) and HE (b) stains in control, 3% DSS-treated, and DSS-treated xanthoangelol-administered mice. DFS: Direct Fast Scarlet, HE: Hematoxylin-Eosin, 3% DSS: 3% dextran sulfate sodium.
The treatment with 3% DSS for 7 days reduced the length of the colon. These results demonstrated that DSS induced colonic atrophy. However, the administration of xanthoangelol (25 mg/kg) markedly ameliorated DSS-induced colon atrophy (Fig. 3a and 3b). As shown in Fig. (3a), normal stool condition into colon could not clearly be observed among DSS-treated group (control) and DSS-treated plus xanthoangelol (10 and 25 mg/kg) groups. As shown in colonic photographs of several reports [20-23], normal stool conditions could not be clearly observed among photographs in the DSS-treated group, DSS plus various natural product-treated groups and DSS plus a positive control drug 5-aminosalicylic acid-treated group.

Fig. (4) shows the histology of the DFS- and HE-stained colon. The treatment with 3% DSS for 7 days induced mucosal ulceration (Fig. 4a and 4b) and eosinophil infiltration (Fig. 4a). Xanthoangelol (10 and 25 mg/kg) attenuated these DSS-induced changes (Fig. 5).

3.4. Colonic MCP-1, IL-1β and TNF-α Levels in DSS-Treated Mice

The treatment with 3% DSS for 7 days increased colonic MCP-1, IL-1β and TNF-α levels (Fig. 6). The increases observed in MCP-1 and IL-1β levels were significantly attenuated by xanthoangelol (25 mg/kg) (Fig. 6a and 6b), while those in colonic TNF-α levels were significantly reduced by both doses of xanthoangelol (Fig. 6c).

Fig. (5). Effects of xanthoangelol on leukocyte infiltration to the colonic mucosa (a) and colonic mucosal layer (b) in 3% DSS-treated mice. Values are the means ± SEM of six mice. * Significantly different from 3% DSS-treated mice. P<0.05. DSS: 3% dextran sulfate sodium.

Fig. (6). Effects of xanthoangelol on colonic MCP-1, IL-1β, and TNF-α levels in 3% DSS-treated mice. Values are the means ± SEM of six mice. * Significantly different from 3% DSS-treated mice. P<0.05. MCP-1: monocyte chemoattractant protein 1, IL-1β: interleukin 1β, TNF-α: tumor necrosis factor-α, DSS: 3% dextran sulfate sodium.
4. DISCUSSION

IBD [7-10] frequently causes diarrhea, which has a negative impact on the quality of life of patients. *A. keiskei* herbs and roots have been used to treat the abdominal pain caused by diarrhea. The present study investigated the effects of xanthoangelol, a major chalcone in *A. keiskei*, on the symptoms associated with IBD, such as bloody stools and reduced food intake, in DSS-treated mice. We found that xanthoangelol (10 and 25 mg/kg) ameliorated DSS-induced increase in leukocytes counts and decreased in red blood cell and platelet counts as well as Hb and Ht levels. Recently, it was reported that Leukocytaphresis (LCAP), a method that removes leukocytes (granulocytes, monocytes and macrophages) from the circulatory system, and the majority of patients with ulcerative colitis treated with LCAP achieved clinical remission [24, 25]. It seems likely that the increase of leukocyte counts in the blood may be associated with inflammation of colon tissue by the treatment of DSS drinking water. The spleen weight had no effect by DSS-treatments, but the thymus weight reduced by DSS-treatment. Xanthoangelol (25 mg/kg) inhibited the reduction of thymus weight by DSS treatment (Table 3). This result may be associated with immune-reaction, but unknown; therefore, further studies are needed to clarify the immune-function by the isolation of thymus T cells. Recently, Wang *et al.* [26] reported that averitin (an anesthetics in rodent experiments) is widely euthanized in Inflammatory Bowel Disease (IBD) model, and that it increases the number of neutrophils and macrophages, and neutrophil-related Myeloperoxidase (MPO) production, consequently averitin aggregative inflammation in early and middle stages on DSS-induced colitis. Xanthoangelol (25 mg/kg) prevented the aggravation of stool condition, bleeding stool and DAI on days 3-5 (middle stage) by DSS treatment, but not last stage (days 6, 7) (Fig. 2). Further, xanthoangelol (25 mg/kg) inhibited the elevations of colon cytokines (IL-1β and TNF-α) and a chemokine (MCP-1) by DSS treatment. These findings suggest that xanthoangelol may be due to the inhibition of colon inflammation at the middle stage through increasing colon cytokines and a chemokine produced by colonized activated macrophages in DSS-treated mice. There are many reports regarding the actions of natural products on neutrophils, T cells and macrophages in DSS-treated colitis [27-31]. Further studies are needed to clarify the relationship among neutrophil, macrophage and T cell invasion to colon mucosa by immune-histochemical observation in DSS treatment. Histological analysis revealed that xanthoangelol (10 or 25 mg/kg) prevented DSS-induced mucosal ulceration and eosinophil infiltration. Previous studies reported a close relationship between eosinophils and IBD [32-35]. Smyth *et al.* reported that activated eosinophils were associated with enteric nerves in IBD patients [36]. It is well-known that eosinophils release the major basic protein (MBP), and that MBP causes the interference to normal cell function [37]. This finding indicates that xanthoangelol may partly protect against DSS-induced colon inflammation and/or damage by suppressing eosinophil infiltration of the mucosal membrane. Further studies are needed to clarify the role of colon eosinophils in DSS-treated mice.

These results indicate that xanthoangelol ameliorated DSS-treated colitis by suppressing increases in the inflammatory chemokine MCP-1 and cytokines IL-1β and TNF-α. Rosmarinic acid (phenolic ester compound) [23] and xanthohumol (chalcone derivative) [28] prevent DSS-induced colitis via the inhibition of NF-kB and Signal Transducer and Activator of Transcription 3 (STAT 3) activation (in vivo), and IKKβ/NF-κB signaling pathway (in vitro and in vivo), respectively. *In vitro* and *in vivo* studies that investigate the mechanisms of action of xanthoangelol using macrophages or T cells in DSS-treated colitis are needed in detail.

CONCLUSION

The present results demonstrated that xanthoangelol, isolated from the roots of *A. keiskei*, inhibited eosinophil infiltration of the colon and suppressed increases in the levels of the inflammatory chemokine MCP-1 and cytokines IL-1β and TNF-α in mice treated with DSS. Based on these results, xanthoangelol has potential as a treatment for IBD. Further, both *in vitro* and *in vivo* studies are needed to investigate the mechanisms of action of xanthoangelol using macrophages or T cells isolated from DSS-treated colitis tissues.

AUTHOR CONTRIBUTIONS

Dr. Y. Kimura designed the experiments, conducted all experimental work, wrote the manuscript, and held discussions. Honorary Prof. K. Baba carried out the isolation of and supplied xanthoangelol. Y. Kimura and K. Baba read and approved the final manuscript.

LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>CD</td>
<td>Crohn’s Disease</td>
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<tr>
<td>DAI</td>
<td>Disease Activity Index</td>
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<tr>
<td>DFS</td>
<td>Direct Fast Scarlet</td>
</tr>
<tr>
<td>DSS</td>
<td>Dextran Sulfate Sodium</td>
</tr>
<tr>
<td>IBD</td>
<td>Inflammatory Bowel Disease</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>MCP</td>
<td>Monocyte Chemoattractant Protein</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate-Buffered Saline</td>
</tr>
<tr>
<td>UC</td>
<td>Ulcerative Colitis</td>
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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All experiments confirmed to and were approved by the Ethics Committee on Animal Experimentation, Ehime University, Japan (approval no. YA-1-1).

HUMAN AND ANIMAL RIGHTS

No humans were used in experiments that were the basis of this research. All experiments performed on mice conformed to the Ethical Guidelines for Animal Experimentation, Ehime University, the Japanese Pharmacological Society, and the Guide for the Care and Use of Laboratory Animals by the National Institute of Health, Japan.

RESEARCH INVOLVING ANIMALS

Research involving animals was performed according to the Ethical Guidelines for Animal Experimentation, Ehime
REFERENCES


[24] Krznarić, Ž.; Markoš, P.; Goličič Čepulić, B.; Čuković-Cavka, S.; Domislović, V.; Bojanić, I.; Barišić, A.; Kekez, D. Leukocyctapher-


