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Comparison of Oral versus Rectal Administration of Processed-*Scutellaria baicalensis* on Colonic Inflammation in Mice

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ABSTRACT – We previously showed that the water extracts of rice wine-baked *Scutellaria baicalensis* Georgi (RWBS) ameliorated colonic inflammation more than crude *Scutellaria baicalensis* (CS) after oral administration. The aim of this study is to compare the effect of rectal and oral administration of RWBS in the experimental colitis. Experimental colitis was induced in mice by daily treatment with 5% dextran sulfate sodium (DSS) in the drinking water for 7 days. Water was used as vehicle of oral administration, while Carbopol/PEG mucoadhesive gel was used as vehicle of rectal administration. RWBS and RWBS gel (RWBSG) were administered once per day for 7 days. RWBS and RWBSG significantly attenuated the disease activity index (DAI) calculated as the sum of scores of body weight loss, stool consistency and rectal bleeding. Furthermore, RWBS and RWBSG reduced the mucosal myeloperoxidase activity and COX-2 (cyclooxygenase-2) expression in colon tissue. Anti-inflammatory effect of CS on colonic inflammation was increased by baking with rice wine in both oral and rectal administration. Moreover, anti-inflammatory effects of oral administration on colonic inflammation was greater than those of rectal administration. Further study would be required for the development of intra-rectal formulation.

Key words- *Scutellaria baicalensis,* Colitis, Mucoadhesive gel, Inflammation

Ulcerative colitis (UC) is an inflammatory disease of unknown cause that exhibits an unpredictable clinical course with remission and relapsing exacerbations, characterized by rectal bleeding and diarrhea.[1)] Large numbers of neutrophils and macrophages pass out of the circulation and enter the inflamed mucosa and submucosa of the large intestine during acute inflammation, and an increase of myeloperoxidase (MPO) activity and increases of TNF-α and COX-2 expression have been observed in the mucosa of ulcerative colitis in humans and experimental animals.[2)] An experimental colitis displaying morphological changes similar to those of human UC can be induced in mice through drinking water containing dextran sulfate sodium (DSS). Most prevailing therapies for UC include aminosalicylates, glucocorticosteroids and immunomodulators. However, they display limited beneficial action because of a variety of systemic adverse reactions.[3,4)]

Interest on traditional Chinese herbal medicine has recently been increased for the treatment of these disorders. Root of *Scutellaria baicalensis* contains a large number of flavonoids, baicalin, baicalein, and wogonin have been extensively explored to show anti-inflammatory activity,[5,6)] antiallergic activity,[7)] antioxidative effect,[8)] antiviral effect[9)] and antigenotoxic effect.[10)] Oren-gedoku-to (Huang-Lian-Jie-Du-Tang) including *Scutellaria baicalensis* was reported to reduce the symptoms of colitis in DSS-induced colitis.[11)] We previously found that the rice wine-baked *Scutellaria baicalensis* (RWBS) has more anti-inflammatory effect than those of crude *Scutellaria baicalensis* (CS) on DSS-induced colitis in mice.[12)]

As UC primarily represents a local inflammation of the mucosa in the colon and in parts of the small bowel, it would be ideal to target the therapeutic principles directly to the affected areas. Recently, there has been increasing interest in the development of new formulations that can control the release of drugs using mucoadhesive polymer. Mucoadhesive polymers have been used to develop nasal, rectal, gastrointestinal drug delivery.[13,14)] Carbopols, which are very high molecular weight polymers of acrylic acid, have been used mainly in liquid or semi-solid pharmaceutical formulations, such as gels, suspensions and emulsions. PEG 400 increases their thickening behaviour after a thermal treatment, and respond better to the rheological definition of gel.[15)]

In the present study, a Carbopol 971/PEG 400 mucoadhesive gel containing the water extract of RWBS was prepared for rectal administration. Anti-inflammatory effects after intra-rec-

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tal administration of RWBS were compared with those after oral administration on experimental colitis induced in mice with DSS.

Materials and Methods

Chemicals

Baicalein and baicalin were purchased from Aldrich Chem. Co. (Milwaukee, WI, USA). The HPLC grade chromatographic solvents were obtained from J.T. Baker (Mallickrodt Baker Inc., USA). Double distilled water was used for all the preparations.

Animal

Specific pathogen-free female BALB/c mice (7 weeks old at the start of the experiments) were obtained from the Damool Science animal facility (Daejeon, Korea). The animals were housed in a specific pathogen-free environment, and standard chow pellet diet and drinking water were provided freely.

Preparation of RWBS and RWBSG

Root of *Scutellaria baicalensis* Georgi (Labiatae, CS) was purchased from the Oriental drug store, Daehak Hanyakkuk (Iksan, Korea). Rice wine-baked *Scutellaria baicalensis* (RWBS) was prepared to mix with 10 g rice wine per 100 g CS standing for 2 h with capping tray, and to bake in heated parching machine. CS and RWBS have been deposited at the Herbarium at the College of Pharmacy, Wonkwang University. Each water extracts were prepared by decocting for 2 h with 1L distilled water per 100g CS. The extract was filtered through a 0.45 μm filter, lyophilized, and kept at 4°C. The dried extract was dissolved in sterile water before use. The Carbopol/PEG hydrogels (ASAG, CSG or RWBSG) containing 5-Aminosalicylic acid (ASA), CS or RWBS were prepared by professor Hoo-Kyun Choi at the College of Pharmacy, Chosun University.

Determination of Baicalin and Baicalein in RWBS

The chromatographic system consisted of Communications Bus Module (CBM-10A) autoinjector (SIL-10A), Diode array detector (CBM-M10A VP), a liquid chromatograph (LC-10AT) (Shimadzu, Kyoto, Japan). A ìBondapak C18 column (300×3.9 mm, 10 μm particle size, Waters Chromatography Division, Milford, MA) was used. The mobile phase was methanol-acetonitrile-water (10:28:62) and pH adjusted to 3.5 with phosphoric acid, filtered through a 0.45 μm filter and degassed prior to use. The flow-rate was 1 mL/min. Detection was performed at a wavelength of 280 nm at room temperature. A 20 μL volume of sample was injected for each separation. The dried extracts (0.5 g) of CS and RWBS were mixed individually with 10 mL water on vortex for 5 min. Then each sample was separated by centrifugation at 4000 rpm for 10 min. The supernatant was injected onto HPLC for analysis. All extracts were filtered through a 0.45 mm membrane filter before injecting into the HPLC system. A 20 μL volume of sample solution was injected onto HPLC for analysis.[16)]

Induction of Experimental Colitis and Administration of RWBS and RWBSG

DSS (mol wt 36,000-50,000) was obtained from ICN Biomedicals (OH, USA) and dissolved in distilled water. Colitis was induced by providing drinking water ad libitum containing 5% DSS (w/v) from on day 0 for 7 days. Mice were divided into the following eight groups and they were weighed every other day: a water group (group I), a DSS group (group II), a DSS with [1]ASA (group III, 100 mg/kg), a DSS with [2]ASAG (group IV, 200 mg/kg), a DSS with [3]CS (group V, 1g/kg), DSS with [4]CSG (group VI, 2 g/kg), DSS with [5]RWBS (group VII, 1 g/kg), DSS with [6]RWBSG (group VIII, 2 g/kg). Water extracts and gels were administered once per day for 7 days. The treatments were started on day 0 and stopped on day 7. ASA, therapeutic drug of colitis and CS were used as control. In this model, mice were checked daily for body weight and stool consistency.

Disease Activity Index (DAI)

Total body weight, blood in the stool and stool consistency were determined daily and classified according to a 0-4 point-scale as shown in Table I. The appearance of rectal bleeding was defined: 0 = no bleeding, 2 = occult bleeding, 4 = gross macroscopic bleeding (blood around the anus or in the cage). Normal stool was formed pellets = 0; loose stool was pasty and semiformed which did not stick to the anus = 2; diarrhea was liquid stools that stuck to the anus = 4. The clinical disease activity index (DAI) was calculated as the sum of scores for weight loss, stool consistency and blood in feces. DAI is presented as daily changes and as the sum of scores for the complete experimental period.

[1]5-Aminosalicylic acid
[2]5-Aminosalicylic acid Gel
[3]Crude *Scutellaria baicalensis*
[4]Crude *Scutellaria baicalensis* Gel
[5]Rice wine-baked *Scutellaria baicalensis*
[6]Rice wine-baked *Scutellaria baicalensis* Gel

Table I–*Disease Activity Index*

Score	Weight loss (%)	Stool* consistency	Occult/gross bleeding
0	(-)	Normal	Normal
1	1 - 5		
2	5 -10	Loose	Guiac (Occult)
3	11-15		
4	> 15	Diarrhea	Gross bleeding

The DAI is calculated as the sum of scores of weight loss, stool consistency and bleeding. *Normal stool = well formed pellets; loose stool = pasty and semiformed stool which do not stick to the anus; diarrhea = liquid stools that stuck to the anus.[17]

Western Blot Analysis

For analysis of expression 100 mg of distal colon were homogenized in 600 μL of lysis buffer (iNtRON Biotech) on ice. The lysates were incubated for 30 min on ice or freezer at -20°C. The lysates were centrifuged at 13,000 rpm for 5 min and supernatants were transferred to a fresh tube. Protein concentration was determined using a PRO-MEASURE solution (iNtRON Biotech), and 50 μg of protein was separated by 10-15% SDS-PAGE and transferred to PVDF membrane (Hybond-PVDF, Amersham Pharmacia Biotech, Piscataway, NJ, USA). After blocking with 5% skim milk, membranes were incubated with COX-2 antibody for 12 h at 4°C. After washing the membranes in PBS containing 0.1% polyethylene-sorbitan monolaurate (Tween 20), the immunoblots were incubated with the secondary antibody, goat anti-mouse IgG-horseradish peroxidase, at a 1:20,000 dilution for 1 h at room temperature. Finally, epitopes on proteins recognized specifically by antibodies were visualized by using enhanced chemiluminescence (ECL) detection kit (Amersham, Milan, Italy).

Assay for Myeloperoxidase (MPO) Activity

MPO was extracted from the homogenized esophageal tissues by suspending the materials in 0.5% hexadecyltrimethyl ammonium bromide (Sigma, St. Louis, MO, USA) in a 50 mM potassium phosphate buffer, pH 6.0 before sonication on ice bath and then centrifuged at 3000 rpm for 20 min at 4°C. For the assay of myeloperoxidase activity, the following reagents were added to wells of a 96-well microtiter plate. The supernatant (50 μL) was mixed with 50 μL of 50 mM phosphate-buffered solution containing 0.5% HTAB (pH 6.0), 50 μL o-dianisidine (0.68 mg/mL in distilled water) (Sigma), and 0.003% hydrogen peroxide. The change in absorbance was measured spectrophotometrically at 450 nm. Pure human MPO was used as a standard (Sigma). The inhibition percentage of MPO activity was calculated using the following equation:

$$\text{Inhibition}(\%) = \frac{(A-B)}{A} \times 100$$

Where A is MPO activity in DSS-treated mice without the water extracts or gels, and B is MPO activity in mice with the water extracts or gels.

Statistic Analysis

Values were expressed as mean±S.E. Statistical significance was determined using the Student's t-test to express the difference between two groups.

Results and Discussion

Contents of Baicalin and Baicalein in RWBS

To determine the anti-inflammatory ingredients in the water extracts of RWBS, HPLC analysis was carried out. As shown Fig. 1, the content of baicalin (2.1±0.2%) and baicalein (0.35±0.03%) in RWBS was higher than those (0.9±0.1% and 0.05±0.01%) in CS. The increase rate of extraction for baicalein was greater than that for baicalin. The aim of processing is to increase efficacy or to reduce toxicity of traditional herbal medicines. This result suggests that baicalein, an active ingredient from *Scutellaria baicalensis,* might easily be extracted by baking with rice wine.

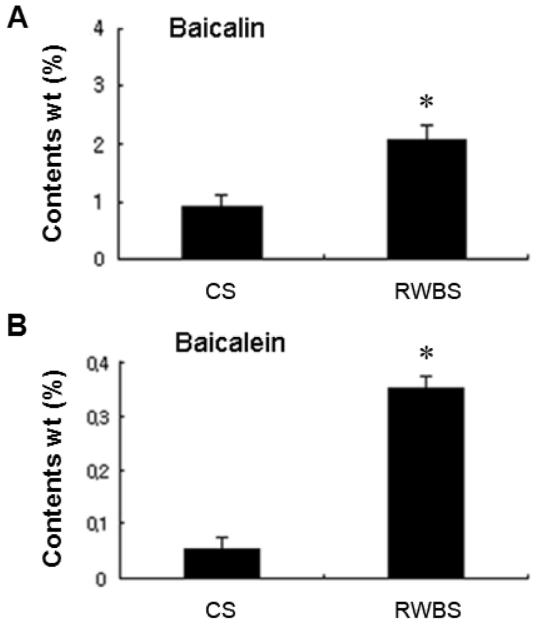

Figure 1–Contents (wt%) of baicalin(A) and baicalein(B) content in the water extract of CS or RWBS. Data represent mean±SD (*P<0.05 vs CS, n=6).

Effect of RWBS or RWBSG on Colon Length and DAI

DSS colitis mice administered the water extracts or gels were monitored daily for clinical sign for 7 days. All animals survived until sacrificed on day 7. On day 7, all mice treated with 5% DSS showed significant body weight loss (data not shown) or colon shortening (Fig. 2), while administration of water extracts or gels significantly reduced the body weight loss or colon shortening of colitis mice compared with water group (Fig. 2). Inhibitory effect of RWBSG was greater than those of CSG, while it was smaller than those of ASAG.

In DSS-treated mice, the DAI increased progressively, reaching 3.5 at day 7 (Fig. 3). DAI of RWBSG, CSG and ASAG were 1.3, 2.1 and 1.1 and its inhibition rates on DAI were 62.9%, 40%, and 68.6%, respectively (Fig. 3). The anti-inflammatory effect of *Achyranthes bidentata* processed with rice wine was better than that of crude *Achyranthes bidentata*.[18] We previously showed that RWBS significantly inhibited the DSS-induced colitis after oral administration.[12] Lin *et al.* showed that in DSS-induced colitis, baicalein was more effective than baicalin because baicalin was absorbed more slowly and to a lesser extent than baicalein.[19] These results indicates that increment of baicalein content in RWBS by processing with rice wine might result in inceased anti-inflammatory effect.

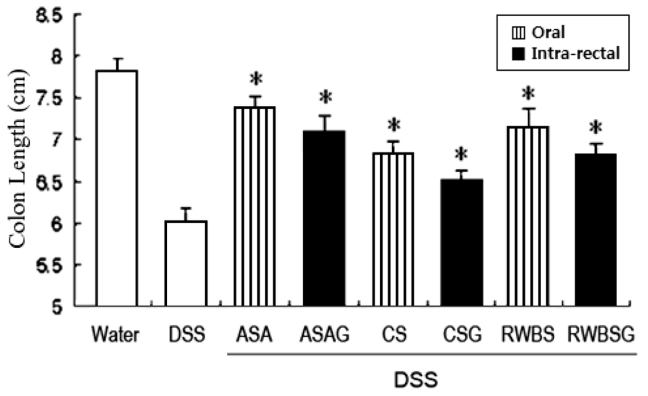

Figure 2–Effect of RWBS (1 g/kg) or RWBSG (2 g/kg) on colon length in DSS-induced colitis. RWBS was orally administered (▥) and RWBSG was rectally administrated (■) throughout the treatment period with DSS. ASA (100 mg/kg), ASAG (200 mg/kg), CS (1 g/kg) or CSG (2 g/kg) were used as control. Data were expressed as the means ± S.E.M (*P<0.05 vs DSS, n=10).

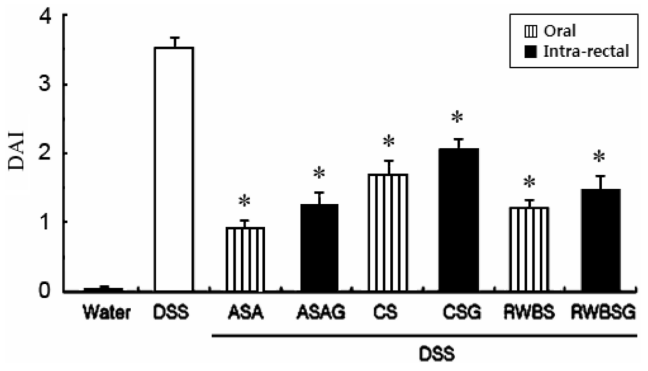

Figure 3–Effect of RWBS (1 g/kg) or RWBSG (2 g/kg) on DAI in DSS-induced colitis. RWBS was orally administered (▥) and RWBSG was rectally administrated (■) throughout the treatment period with DSS. ASA (100 mg/kg), ASAG (200 mg/kg), CS (1g/kg) or CSG (2g/kg) were used as control. The DAI data were calculated as shown in Table I and represented as the mean values (*P<0.05 vs DSS, n=10).

Effect of RWBS or RWBSG on COX-2 Expression

The proinflammatory protein such as COX-2 has a key role in the pathogenesis of IBD. The colonic expression of COX-2 was measured by Western blot analysis. Although a faint band was detected in the control group, an intense band was observed in DSS group. In contrast, pretreatment with ASAG inhibited COX-2 expression to normal level. Although RWBSG markedly reduced COX-2 expression, CSG slightly reduced (Fig. 4). It has been known that baicalein has inhibitory effect on the production of inflammatory cytokines[20] and baicalin exhibits anti-inflammatory activity by binding to chemokines to limit their biological function.[21] This result shows that baicalin and baicalein in RWBS might inhibit the COX-2 expression in colon tissue.

Effect of RWBS or RWBSG on MPO Activity

MPO activity correlates closely with clinical, macroscopic, and histological grading of intestinal inflammation in the experimental groups. The MPO activity in normal mice was 1.77±0.43 units/g tissue, whereas the MPO activity in DSS-

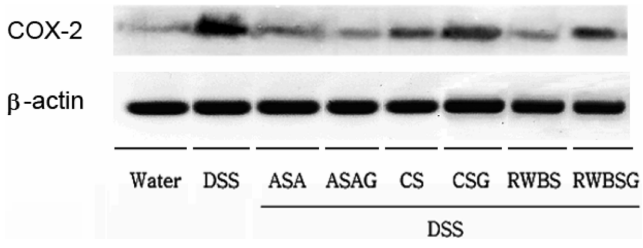

Figure 4–Effect of RWBS (1 g/kg) or RWBSG (2g/kg) on COX-2 expression in colonic tissues. RWBS was orally administered and RWBSG was rectally administrated throughout the treatment period with DSS. ASA (100 mg/kg), ASAG (200 mg/kg), CS (1 g/kg) or CSG (2g/kg) were used as control. Production of COX-2 protein was determined by Western blot analysis as described in materials and methods.

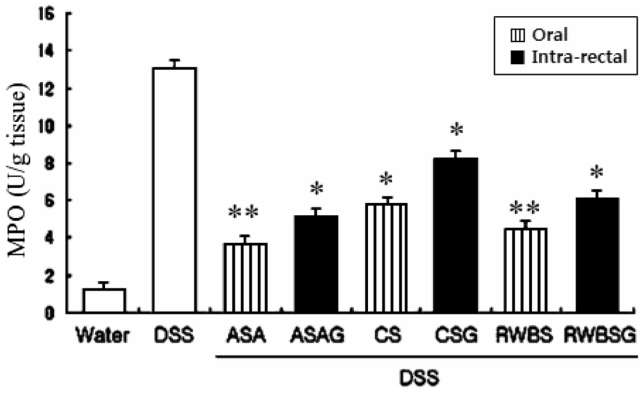

Figure 5–Effect of RWBS (1 g/kg) or RWBSG (2 g/kg) on MPO activity in colonic tissue. RWBS was orally administered (▥) and RWBSG was rectally administrated (■) throughout the treatment period with DSS. ASA (100 mg/kg), ASAG (200 mg/kg), CS (1 g/kg) or CSG (2 g/kg) were used as control. MPO activity was determined as described in Materials and Methods. The data are expressed as the means±S.E.M. (*P<0.05, **P<0.01 vs DSS, n=10).

treated mice was increased to 13.045±0.370 units/g tissue. In contrast, the tissue content of MPO in ASAG, CSG, or RWBSG group was found to be 5.66±0.22 units/g, 8.12±0.76 units/g or 6.02±0.42 units/g, respectively. MPO activity was inhibited by at least 56.6%, 37.7% or 53.9% (Fig. 5). Neutrophils are known to cause tissue damage by releasing toxic products such as MPO in colon mucosa of patients with colitis. A report showed that baicalin and baicalein significantly impeded the reactive oxygen intermediates production by peripheral human leukocytes.[22] It may be suggested that inhibitory action of the water extracts or gels on colitis is attributable to inhibition of neutrophil infiltration into the colonic mucosa.

Conclusions

In this study, in both oral administration and intra-rectal administration, anti-inflammatory effect of *Scutellaria baicalensis* on DSS-induced colitis was increased by baking with rice wine. Anti-inflammatory effect on colonic inflammation after oral administration was greater than that after rectal administration. It is likely that active ingredients of RWBS remained in the Carbopol/PEG gel or wasn't absorbed into colonic mucosa. Further study would be required for the development of other intra-rectal formulation that effective drug concentrations can be archived in the colonic mucosa and systemic availability can be limited to reduce the potential for adverse effects.

Acknowledgements

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