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RESEARCH ARTICLE

Protective effects of *Ulmus macrocarpa* on experimental colitis mice models

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Abstract In traditional Korean medicine, *Ulmus macrocarpa* Hance is a frequently used herb in South Korea for treating intestinal disorders such as colitis. This study investigated whether water extract of *Ulmus macrocarpa* Hance (UME) could show a protective action on 2 different mice models of experimental colitis induced by dextran sulfate sodium (DSS) and 2,4,6-trinitrobenzene-sulfonic acid (TNBS), which have been widely used as inflammatory bowel disease models. Colitis was induced by DSS and TNBS in balb/c mice, respectively. UME at doses of 100, 300, or 1000 mg/kg was orally administered twice a day for 7 d in the DSS model and at doses of 300 or 1000 mg/kg for 3 d in the TNBS model. The body weight of the mice and clinical score were measured daily. Colon length and macroscopic score were assessed on day 7 in the DSS model and on day 3 in the TNBS model. In the DSS model, UME inhibited shortening of colon length and macroscopic damages of the colon, and showed improvement of clinical score, however it did not inhibit weight loss. In the TNBS model, UME did not inhibit weight loss and shortening of colon length. The current results indicate that UME ameliorates DSS-induced colitis in mice similar to human ulcerative colitis, not TNBS-induced colitis in mice similar to human Crohn's disease. Further investigations to unveil the exact mechanisms are needed.

Keywords *Ulmus macrocarpa* · Inflammatory bowel disease · Dextran sulfate sodium · 2,4,6-Trinitrobenzenesulfonic acid · Colitis

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Introduction

Inflammatory bowel disease (IBD), such as Crohn's disease (CD) and ulcerative colitis (UC), is a chronic relapsing disorder of the intestine due to dysregulation of innate and adaptive immune response to intestinal micro-organisms (da Silva et al. 2010). There is an increasing incidence of IBD in the Asia-Pacific region (Ahuja and Tandon 2010). Although current treatments including steroids and non-steroidal anti-inflammatory drugs have shown remission of the symptoms, they have some limitations like allergy, nausea and lymphoma (Siegal 2011). Therefore, many researches about medicinal plants for treatment of IBD and as alternative agents of conventional treatments have been conducted (Lee et al. 2009).

Ulmus macrocarpa Hance (Ulmaceae; UM) is widely distributed in Korea, Japan and China. The stem bark has been used as heat-clearing and detoxicating medicinal to treat dermatitis, gastric cancer, mastitis, constipation, dysuria, hematuria and edema in traditional Korean

medical clinics (Oh et al. 2008). Recently, treatment of arthritis as herbal acupuncture is in the limelight of clinicians (Lee et al. 2008). Until now, various pharmacological effects of UM such as anti-microbial activitiy (Park et al. 1999), anti-analphylactic action (Kim et al. 1998), inhibition of nitric oxide-production and matrix metalloproteinases (Song et al. 2003; Kim et al. 2004), anti-ulcer action in stomach (Lim and Cui 2002), anti-hypertensive action (Oh et al. 2008) and anti-cancer activity (Yoon et al. 2009) have been reported. However, as far as we know, there is no study about the effects of UM on IBD models.

There are several methods to induce IBD-like colitis in animals. Particularly, dextran sulfate sodium (DSS) and 2,4,6-trinitrobenzene sulfonic acid (TNBS) have been widely applied chemicals to induce murine colitis (Alex et al. 2009). We hypothesized that UM might show protective effects on the IBD animal models. In the current study, we investigated the effects of UM by measuring body weight, colon length, macroscopic score and clinical score at 7 d after DSS treatment and at 3 d after TNBS treatment in mice.

Materials and methods

Chemicals and drugs

DSS (Molecular weight 36000–50000 kD, MP Biomedicals, Japan), TNBS (Sigma-Aldrich, USA) and isoflurane (Abbott Lab Ltd, USA) were used in the experiment. DSS was dissolved in filtered drinking water for tapping water. TNBS was mixed with 50% ethanol before anal enema.

Animals

Balb/c mice (22–23 g) were obtained from Daehan Bio Link (Seoul, Korea). Animals were housed at an ambient temperature of 20–22°C and 50±10% humidity and freely allowed food and water ad libitum. All mice were acclimated a week before experiment. All experimental procedures were conformed to the international guidelines ‘Principles of Laboratory Animals Care’ (NIH publication no. 85–23 revised 1985 and Kyung Hee University 2006). The international animal ethical committee of Kyung Hee university approved the experimental protocol (KHUASP (SE)-09-036).

Preparation of extract

Dried stem bark of UM was purchased from Kyung Hee Hanyak Co (Seoul, Korea). One hundred ninety gram of sample was extracted with hot water (100°C) for 2 h, filtered, and freeze-dried to a powdered form (PS004, 3.81 g, yield=1.59%). The extract specimen of UM (UME) was deposited in the herbal laboratory of the college of Oriental Medicine, Kyung Hee University.

Induction of colitis by dextran sulfate sodium (DSS)

The male balb/c mice were provided with drinking water containing 5% DSS ad libitum for 7 d. The mice were sacrificed on day 7 of the experiment. UME was administered twice a day by feeding tube during each experiment. Mice were divided into 5 groups including normal, control, and UME-treated (100, 300, or 1000 mg/kg twice a day, p.o.) groups (n=8, each).

Induction of colitis by 2,4,6-trinitrobenzenesulfonic acid (TNBS)

Male balb/c mice were anesthetized by isoflurane (2%), and later 100 μl of 2.5% TNBS in 50% ethanol solution was injected intrarectally via a 1-ml syringe attached to a 3.5-cm flexible catheter. Three days after the TNBS injection, all mice were sacrificed. UME was administered twice a day by feeding tube in the same manner of DSS experiment. Mice were divided into 4 groups including normal, control and UM-treated (300 or 1000 mg/kg twice a day, p.o.) groups (n=8, each).

Measurement of body weight and colon length

In each experiment, the body weight was monitored at 9 a.m. daily, and the whole colonic length was measured immediately after sacrifice.

Scoring of clinical findings

Clinical score was measured by modified method of previous report (Johswich et al. 2009). Two investigators blinded to the groups daily measured the score after DSS treatment. Clinical score was applied as follows: spontaneous behavior and posture (4, moving [+++] without hunching; 3, moving [++] without hunching; 2, moving [+] with hunching; 1, moving [±] with hunching; 0, moving [−] with hunching), coat and piloerection (4, normal state; 3, clean and yellowish [+] without piloerection; 2, yellowish [++] with piloerection [+]; 1, dirty and yellowish [+++] and piloerection [++]; 0, yellowish [light brown] and piloerection [+++]), and cleaning of perianal region (4, normal state; 3, with stool [+] trace; 2, with stool [++] and blood [+] trace; 1, with stool [+++] and blood [++] trace; 0, herniation with blood [+++]). Clinical score was obtained by summing all scores.

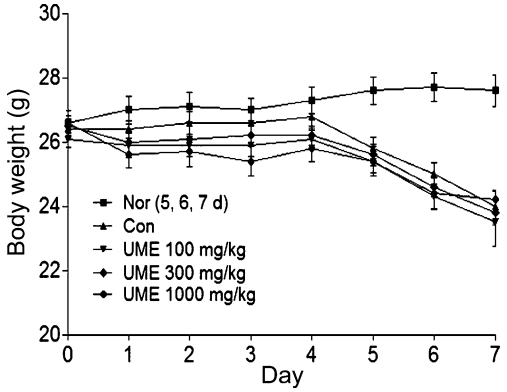

Fig. 1 Effects of UME (100, 300 and 1000 mg/kg) on body weight in DSS-induced colitis. Data represent mean ± SEM. The numbers of parenthesis mean the days on which mice weight of normal group showed significant differences compared to control groups by one-way ANOVA ($P<0.01$). Nor, Con and UME mean normal group, control group and *Ulmus macrocarpa* water extract-treated group, respectively

Scoring of macroscopic findings

Macroscopic score was measured by a modified method of previous report (Hyun et al. 2008). Two investigators blinded to the groups measured the score at day 7 after DSS treatment. The score was applied as follows: edema (4, normal [0.1–0.2 mm in colon thickness]; 3, edema [±, 0.2–0.25 mm]; 2, edema [+, 0.25–0.30 mm], 1, edema [++, 0.3–0.35 mm]; 0, edema [+++, >0.35]) and overall health state (4, no bleeding with normal stool; 3, no bleeding with semiformed stool; 2, fecal blood [+] with pasty and semiformed stool; 1, fecal blood [++] with tar stool [+]; 0, bleeding [+++] with tar stool [++]). Macroscopic score was obtained by summing all scores.

Statistical analysis

All results are expressed as mean ± SEM. Data were analyzed statistically using one-way ANOVA followed by the Dunnett's test. $P<0.05$ was regarded as statistically significant.

Results

Effects on body weight and colon length in DSS-induced colitis

From day 5, the weight of mice in all groups except normal group has shown decreasing tendency. UME did not show protective effect against weight loss caused by DSS (Fig. 1). Shortening of colon length caused by DSS was dose-dependently inhibited in the UME groups (Fig. 2a), especially in UME 300 and 1000 mg/kg-treated group that showed significant inhibitory effects ($P<0.05$ and $P<0.01$, respectively) (Fig. 2b).

Clinical findings and scoring

UME treated groups showed active behavior, healthy appearances and relatively much cleaner perianal lesions compared to control group, however at day 6 and 7, all clinical findings in UME treated groups aggravated similar to control group. UME 100 and 300 treated groups showed improved clinical scores on day 4 compared to control group, especially in UME 1000 mg/kg treated group, improved clinical scores were presented at day 3,4 and 5 ($P<0.05$) (Fig. 3).

Macroscopic findings and scoring

UME treated groups dose-dependently presented lesser edema and fecal blood or tar stool in overall health state compared to control group. UME 300 and 1000 mg/kg treated groups showed significant inhibitory effects of macroscopic scores compared to control group ($P<0.05$ and $P<0.01$, respectively) (Fig. 4).

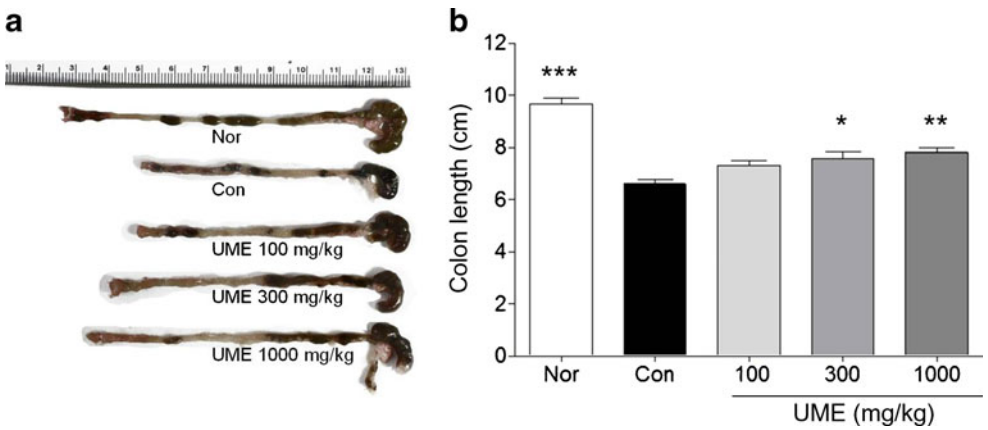

Fig. 2 Typical colon photo of each group (**a**). Effects of UME (100, 300 and 1000 mg/kg) on colon length in DSS-induced colitis (**b**). Data represent mean ± SEM. Nor, Con and UME mean normal group, control group and *Ulmus macrocarpa* water extract-treated group, respectively. * $P<0.05$, ** $P<0.01$ and *** $P<0.001$ versus control group by one-way ANOVA

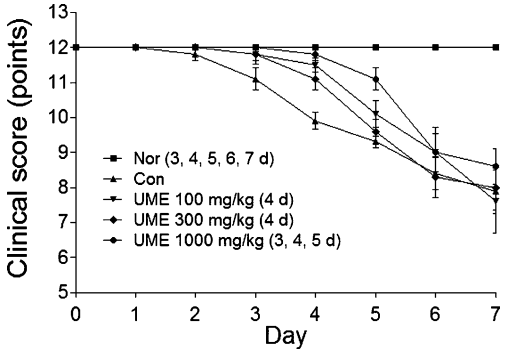

Fig. 3 Effects of UME (100, 300 and 1000 mg/kg) on clinical score in DSS-induced colitis. Data represent mean ± SEM. The numbers of parenthesis mean the days on which clinical score of normal group showed significant differences compared to control groups by one-way ANOVA ($P<0.01$). Nor, Con and UME mean normal group, control group and *Ulmus macrocarpa* water extract-treated group, respectively

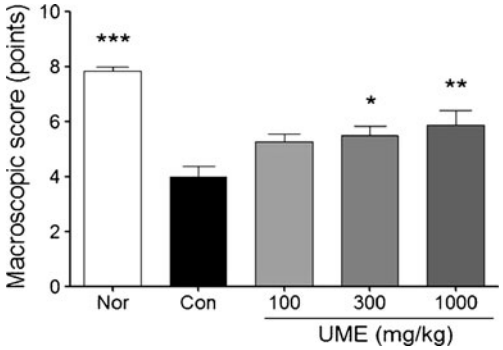

Fig. 4 Effects of UME (100, 300 and 1000 mg/kg) on macroscopic score in DSS-induced colitis at day 7 after induction. Data represent mean ± SEM. Nor, Con and UME mean normal group, control group and *Ulmus macrocarpa* water extract-treated group, respectively. * $P<0.05$, ** $P<0.01$ and *** $P<0.001$ versus control group by one-way ANOVA

Effects on body weight and colon length in TNBS-induced colitis

After TNBS injection, UME treated groups lost weight without any significant differences compared to control group (Fig. 5a). UME 300 and 1000 mg/kg treated groups which have shown significant inhibitory effects in DSS-induced colitis did not exhibit protective effects on TNBS-induced colon shortening (Fig. 5b).

Discussion

In the current study, we demonstrated that UME had protective effects against DSS- but not against TNBS-induced colitis model. In the DSS model, which is widely used in colitis experiments, UME significantly inhibited the shortening of colon length, macroscopic damages and clinical symptoms, but not weight loss. In particular, UME 1000 mg/kg maximally protected DSS-induced colitic damages.

The administration of DSS for 7 d is known to be toxic to gut epithelium, affect the integrity of the mucosal barrier, induce acute colitis in mice and cause weight loss, shortening of colon length, diarrhea, bloody stool, and ulceration of colonic mucosa, similar to the manifestations of human UC (Egger et al. 2000; Maharshak et al. 2010). The most effective dosage of 1000 mg/kg UME twice a day, which translates as 60 g/day twice a day dosage in a human weighing 60 kg is considered to be relatively high for clinical usage. However, this is supported by other reports which reported UME at the dosage of 1000 mg/kg has shown maximal protective effects on 3 different gastric ulcer models (Lim and Cui 2002).

UME dose-dependently improved clinical scores including spontaneous behavior and posture, coat and piloerection, and cleaning of perianal lesion at day 3, 4 and 5, but not day 6 and 7. Clinical score is known to reflect the state of internal organ diseases (Loher et al. 2004; Johswich et al.

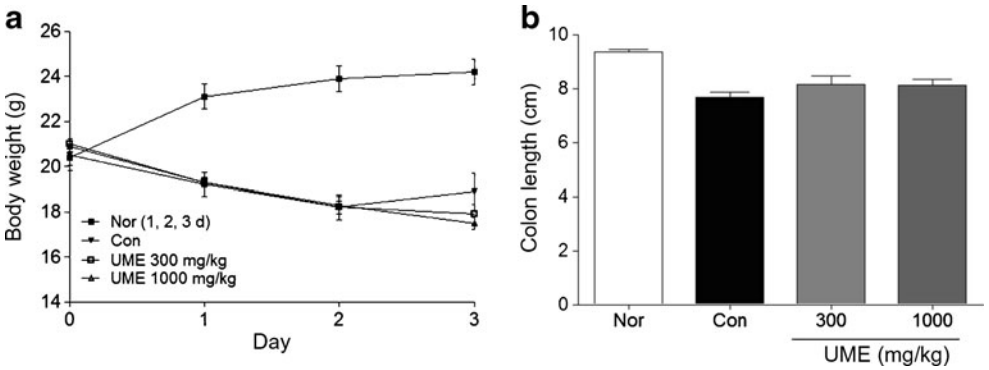

Fig. 5 Effects of UME (300 and 1000 mg/kg) on body weight in TNBS-induced colitis (**a**). The numbers of parenthesis mean the days on which body weight of normal group showed significant differences compared to control groups ($P<0.001$). Effects of UME (300 and 1000 mg/kg) on colon length in TNBS-induced colitis (**b**). Data represent mean ± SEM. Nor, Con and UME mean normal group, control group and *Ulmus macrocarpa* water extract-treated group, respectively

2009). This score could correspond to patient's intestinal or non-intestinal symptoms with colitis. In case of intestinal inflammation including colitis, spontaneous behavior and posture, coat and piloerection, cleaning of perianal lesion, stool consistency and occult blood have been frequently used the assessment of clinical scores (Loher et al. 2004; Johswich et al. 2009). It could suggest that UME improves the symptoms in colitis patients. However, ineffectiveness of clinical scores at day 6 and 7 may originate from severe damages of intestine. It is supported by sudden severe aggravation in mice weight at day 6 and 7. Therefore, from the current results, UME may show the protective effects in not severe colitis.

In the current study, UME dose-dependently improved macroscopic score, which was used to investigate the effects of gross structural damages in colitis. Several studies have shown that macroscopic scores could be another tool to evaluate the severity of inflammation along with histological damages (Rachmilewitz et al. 2002; Hyun et al. 2008). Macroscopic score commonly consists of edema (colon thickness), hemorrhage, ulcer formation, fecal blood, stricture of colon, cecal thickness and overall health state (Rachmilewitz et al. 2002; Hyun et al. 2008). Among them, we used overall health state and edema as macroscopic score. It is considered that the current results of macroscopic score parallel the results of colon length. Therefore, improvement of macroscopic score by UME could be another evidence of the protective effects on intestinal inflammation along with colon length.

However, it did not inhibit weight loss in DSS-induced colitis. DSS administration is known to influence on whole body system. After 7 days of administration, DSS molecules were observed in liver, spleen, kidney, mesenteric lymph node as well as intestine and served as a toxic agent to the tissues (Kitajima et al. 1999). Thus, it could be one of the reasons why UME could not inhibit weight loss in DSS-induced colitis. Moreover, cortex of UM seems to be of little benefit to weight gain in normal state, much less severe colitic state, because it has been used as a heat-clearing and detoxicating medicinal with laxative effect to treat skin diseases, constipation and dysuria (Lee et al. 2008). In addition, the dosage of UME in the current study is very high for clinical usage. Therefore, we could suggest that the effect of UME was local anti-inflammatory effects.

The anti-inflammatory effects of UM have been well established in terms of inhibition of pro-inflammatory cytokines (Prostaglandin E2) or enhancement of anti-inflammatory cytokines (Interleukin-10) (Kim et al. 2004). UM has been also reported to have anti-oxidative, anti-cancer, anti-hypertensive, anti-ulcer and anti-protozoal effects in various pharmacological reports (Park et al. 1999; Lim et al. 2002; Song et al. 2003; Kim et al. 2004; Oh et al. 2008; Yoon et al. 2009). Clinically, it has been reported that oral administration of UM in human UC patients reduced the symptoms of colitis (Ye et al. 1990). These studies supported our current results.

UM have various compounds including 1-heptadecanoyl glycerol, eicosanoic acid, 1-hexadecanoyl glycerol, epifriedelanol, friedelin, camaldulenic acid, arjunolic acid, isoscopoletin and various phenolic acids in the current study (Kim et al. 2004; Wang et al. 2006). We assumed that eicosanoic acid, friedelin and arjunolic acid may be the main compounds in the protective effects of UME (Miles et al. 2002; Ding et al. 2010; Ghosh et al. 2010).

However, UME did not show inhibitory effects on weight loss and shortening of colon length in TNBS-induced colitis model. This model is known to be a well-characterized colitis model, which shows a T helper 1 immune response analogous to the inflammatory course observed in CD, because TNBS is a haptenizing substance in ethanol and cause autologous or microbial proteins to be immunogenic to the host (Maharshak et al. 2010). The cause of ineffectiveness may be because of various factors including severity of colitis and mice species (te Velde et al. 2006).

In conclusion, the current results revealed that UME showed protective effects on human UC-like DSS model but not CD-like TNBS model. Further investigations for possible mechanisms and isolation of active components will be needed.

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