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Protective Effect of Curcumin on Dextran Sulfate Sodium-Induced Ulcerative Colitis in Obese Mice

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Abstract

Ulcerative colitis is a disease that causes inflammation in the mucosal or submucosal layer of the colon. Previous studies have reported that obesity increases the prevalence of ulcerative colitis and aggravates the progression. This study was therefore undertaken to investigate whether curcumin inhibits the progression of ulcerative colitis caused by obesity. Mice were bred on a high-fat diet to induce obesity, and curcumin was administered with a high-fat diet to confirm the anti-inflammatory effect. To induce ulcerative colitis, dextran sulfate sodium (DSS) was administered orally, and clinical symptoms of colitis were subsequently observed. For histological evaluation of curcumin, colon, liver, and abdominal fat tissue samples were prepared and analyzed by hematoxylin and eosin (H&E) and periodic acid-Schiff-alcian blue (PAS) staining. Our results confirm that consumption of curcumin resulted in decreasing the score of the disease activity index, and inhibited shortening of the colon length. In addition, inflammatory cell infiltration and mucosal damage were inhibited in the colon tissue of ulcerative colitis exacerbated by obesity. We further confirmed that exposure to curcumin significantly reduced the steatosis area of the liver and adipocytes of abdominal fat. In conclusion, we believe that curcumin can be applied as a therapeutic agent to treat ulcerative colitis, by inhibiting the progression of colitis caused by obesity.

Keywords

Curcumin
Dextran sulfate sodium
Inflammation
Obesity
Ulcerative colitis

1. Introduction

Ulcerative colitis (UC) is a chronic inflammatory bowel disease of unknown etiology characterized by localized inflammation of the mucosal or submucosal layers of the large intestine, resulting in symptoms such as bloody diarrhea, urgency to pass stools and abdominal pain with episodes of remission and exacerbation [1]. UC is caused by a combination of genetic, immunological, and environmental factors and is most prevalent in North America and Northern Europe [2]. Recently, the incidence has been gradually increasing in southern Europe, Asian countries including Korea, and developing countries [3].

The increasing prevalence of obesity is associated

with an increased incidence of inflammatory bowel diseases (IBDs) such as Crohn's disease (CD) and UC, and there is growing evidence that IBDs are associated with a high-fat, low-fiber Westernized diet [4]. In addition, obesity-induced alterations in gut microbiota composition and stem cell regulation have been reported to promote the development of ulcerative colitis to colorectal cancer [5].

To date, no definitive cure for UC has been developed, and anti-inflammatory drugs, corticosteroids, immunosuppressants, and antibiotics are commonly used in clinical practice, depending on the patient's condition. Although these drugs have some effect in alleviating the course of the disease, they also come with side effects, and new alternative treatments are being actively researched [6].

Curcumin is a polyphenol compound extracted from turmeric and is a biologically active substance with various pharmacological activities including anti-inflammatory, antioxidant, and antiproliferative properties [7]. In India, it is not only used as a food ingredient but also in traditional medicine to combat various chronic inflammatory diseases. Like *Boswellia*, curcumin has anti-inflammatory properties, acting to inhibit inflammatory chemicals produced by immune cells. Curcumin is also an antioxidant and has the ability to remove free radicals from the body [8].

Therefore, in this study, with the aim of finding a drug with alternative therapeutic effects for UC, obese mice fed only on a high-fat diet (HFD) and mice fed with curcumin, the main component of turmeric, along with a high-fat diet, were treated with dextran sulfate sodium (DSS) to induce UC, and the alleviation of colitis by curcumin was analyzed histologically.

2. Materials and methods

2.1. Experimental animals

In this study, 6-week-old C57BL/6J female mice were purchased from KOATECH (Pyungtaek, Korea). The mice were handled in accordance with the Guide for the Care and Use of Laboratory Animals and the provisions of the Animal Welfare Act and the Act on Laboratory Animals. Mice were fed solid food and water ad libitum during the experimental period. The temperature of the animal house was maintained at 21 ± 1°C and relative humidity at 50 ± 2%. The light/dark cycle was 12 hours apart, and the mice were acclimatized for one week before the experiment.

2.2. Experiment design

The 16 rats were divided into two groups (eight in each group), and a different diet was given for each group: high-fat diet (HFD) and curcumin-treated (HFD + CUR). HFD with a fat content of 60 kcal% (ResearchDiets Inc., NZ, USA) was purchased and used, and curcumin (Sigma-Aldrich, MI, USA) was dissolved in carboxymethylcellulose at a concentration of 0.1 g/kg and administered orally daily. In this study, 3% DSS was administered orally for 7 days to induce colitis. The body weight of the rats was measured every three days during the experimental period.

2.3. Disease activity index analysis

To compare the disease activity index (DAI) between the two groups in this study, the rats were scored 0–4 based on their body weight, stool consistency, and gross rectal bleeding (**Table 1**).

2.4. Tissue specimen preparation

Ether-anesthetized mice were laparotomized to remove the colon, liver, and abdominal fat. The large intestine was removed from the cecum to the anus and measured for length, and samples were taken from the distal 5 mm segment and fixed in 10% neutral buffered formalin, pH 7.4. In addition, some tissue from the liver and abdominal fat were fixed equally and paraffin-embedded at a thickness of 4 μm to investigate the association with obesity.

Table 1. DAI scores

Score	Decrease in growth or weight loss (%)	Stool consistency	Occult/gross bleeding
0	0	Normal	Normal
1	1–5	Normal	Occult blood (+)
2	5–10	Loose stools	Occult blood (++)
3	10–15	Loose stools	Occult blood (++)
4	> 15	Diarrhea	Gross bleeding

2.5. Histopathological analysis

Tissue slides of colon, liver, and abdominal fat were deparaffinized in xylene solution, followed by ethanol treatment and water washing, and then subjected to hematoxylin and eosin (H&E) staining, and alcian blue pH 2.5-periodic acid Schiff (AB-PAS) staining for the amount of acidic mucin substances and PAS-positive substances in colon tissue. The area of steatosis was measured in H&E-stained liver tissue to determine the effect of inhibiting lipogenesis, and abdominal fat was calculated as a percentage by measuring the size of adipocytes. The stained tissue slides were observed under a light microscope (Olymphus BX 50, Olymphus Optical Lts., Japan), and images taken with a digital camera (Olympus DP72, Japan) were analyzed using an image analysis program (Image-Pro® Plusver 4.5, Media Cybernetics Inc., GA, USA), and percentages were calculated.

2.6. Evaluation of histological colitis score

According to the degree of inflammatory cell infiltration, the score was evaluated as 0 for no infiltration, 1 for occasional inflammatory cell infiltration in the mucosal lamina propria, 2 for increased inflammatory cells in the mucosal lamina propria, and 3 for aggregated inflammatory cells spreading to the submucosal tissue. In addition, the degree of tissue damage was scored as 0 for no mucosal damage, 1 for isolated lymphoepithelial lesions, 2 for mucosal surface erosions or focal ulceration, and 3 for extensive mucosal damage extending deep into the intestinal wall.

2.7. Statistical analysis

An independent *t*-test was performed for comparison between the two groups, and statistical analysis was performed using SPSS 18.0. A *P*-value of less than or equal to 0.05 was considered statistically significant.

3. Result

3.1. Effect of curcumin on DSS-induced ulcerative colitis in mice

To diagnose DSS-induced UC in mice, clinical symptoms were observed. Severe clinical symptoms, including rectal bleeding, were observed on day 5 in DSS-treated mice (**Figure 1A**). The DAI was compared based on the combined scores of weight change, stool hardness, and rectal bleeding. We found that the HFD + CUR group showed a trend toward decreased DAI and body weight compared to the HFD group (**Figure 1B** and **C**).

3.2. Effect of curcumin on colon length

To assess the effect of curcumin on colon length, the colon of each group was visually observed and measured. Visual observation showed that the HFD + CUR group inhibited the shortening of colon length compared to the HFD group (**Figure 2A**). However, the difference was not significant (**Figure 2B**).

3.3. Effect of curcumin on histological changes in the colon

To analyze the curcumin-induced histological changes in the colon, H&E, and Alcian blue-PAS staining were

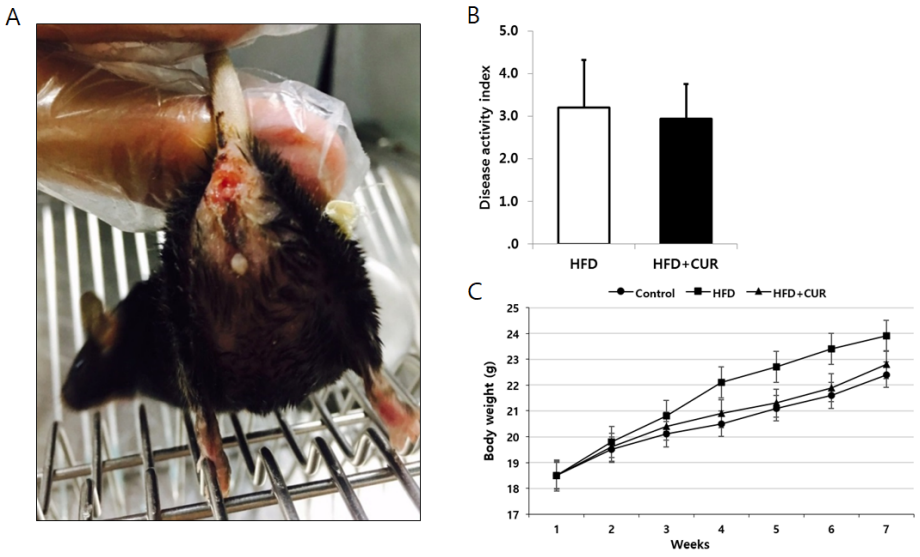

Figure 1. Effect of curcumin (CUR) on DSS-induced UC in mice. (A) Severe clinical symptoms, including rectal bleeding, were observed on day 5 in mice treated with DSS; (B) DAI was evaluated as the average score of body weight changes, rectal bleeding, and stool consistency; (C) Graph of body weight changes. Each value represents the mean ± standard deviation of the mean. *P < 0.05 vs. HFD group, n = 16.

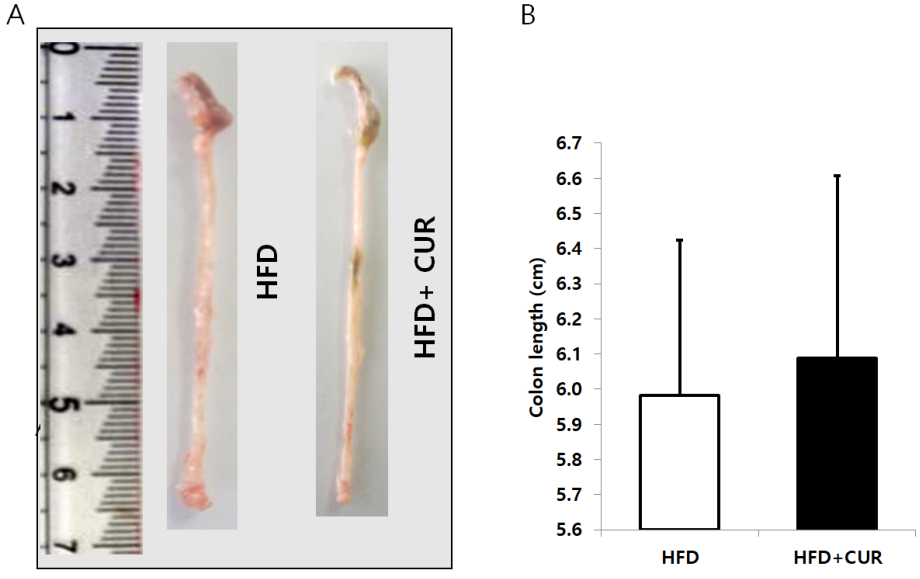

Figure 2. Macroscopic observation and length measurement of the colon. (A) Macroscopic appearance and (B) colon length of each group. Each value is presented as the mean ± standard deviation of the mean, n = 16.

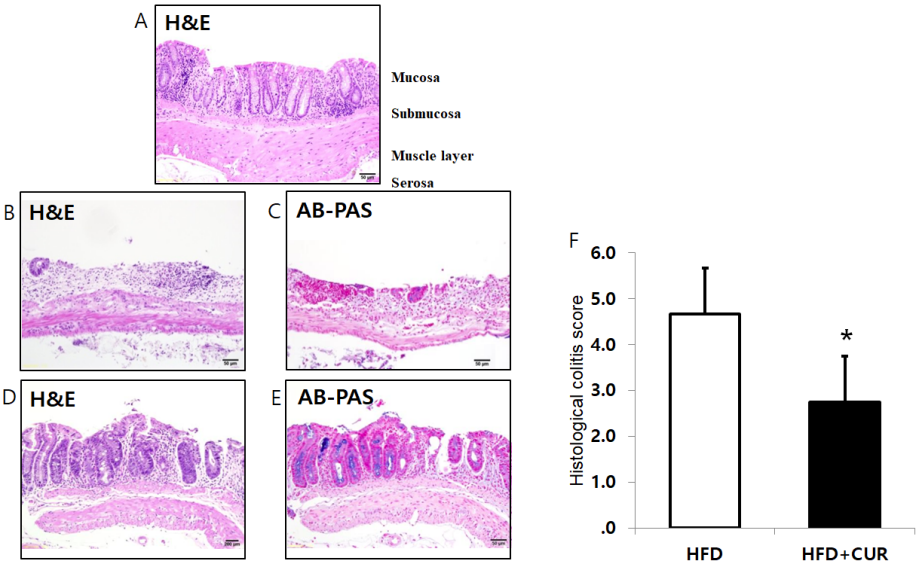

Figure 3. Examination and scoring of histological sections of colonic sections following H&E and Alcian blue-PAS staining. (A) Mice in the normal group; (B, C) mice in the HFD group; (D, E) mice in the HFD + CUR group; (F) histological analysis using a colitis score. Each value is presented as the mean ± standard deviation of the mean. *P < 0.05 vs. the HFD group, n = 16.

performed and evaluated. The colon of normal mice was formed by normal cells with clear boundaries between the mucosal intact layer, submucosa, and muscle layer (**Figure 3A**). However, the mucosal lamina propria of the HFD group showed a significant increase in inflammatory cells, with aggregated inflammatory cells spreading to the submucosa, and a significant decrease in acidic mucus due to mucosal damage caused by dysplasia of the ductal structure (**Figure 3B** and **C**). On the other hand, the HFD + CUR group showed significantly less inflammatory cell infiltration (**Figure 3D**) and almost no mucosal damage (**Figure 3E** and **F**) compared to the HFD group.

3.4. Effect of curcumin on histological changes in liver and abdominal fat

H&E staining was performed to analyze the histological changes in liver and abdominal fat caused by curcumin. In liver tissue, the HFD + CUR group significantly reduced steatosis compared to the HFD group (**Figure 4A** and **B**), and in abdominal fat tissue, the curcumin-treated group significantly reduced adipocytes compared to the HFD group (**Figure 4C** and **D**).

4. Discussion

Ulcerative colitis is a chronic inflammatory bowel disease, for which a variety of drugs are used to alleviate the course of the disease, but many side effects have been reported. Therefore, there is a need for research into alternative treatments for UC. Curcumin has gained attention as a potential osteoarthritis treatment, and its antioxidant and anti-inflammatory properties have been utilized in several therapeutic applications, including digestive disorders, wounds, and rheumatic diseases [9]. In this study, we investigated the effects of curcumin, the main component of turmeric, on the suppression of inflammation and reduction of adipocytes in ulcerative colitis-induced obese mice.

UC was induced by DSS in mice fed with an HFD, which resulted in clinical symptoms of colitis, including rectal bleeding (**Figure 1A**). UC can be characterized by weight loss, loose stools or diarrhea, and symptoms such as occult blood or rectal bleeding [10]. These clinical symptoms were consistent with those of DSS-induced ulcerative colitis in this study. The HFD + CUR group had a reduced DAI compared to the high-fat diet group, suggesting that curcumin alleviated the symptoms of UC (**Figure 1B**).

In a previous study, it was reported that reducing the DAI and inhibiting colon length shortening had a significant effect on alleviating UC [11]. Similarly, in the present study, the HFD + CUR group inhibited colon shortening compared to the HFD group (**Figure 2**). Therefore, it was confirmed that curcumin has a significant effect on alleviating the clinical symptoms of UC and inhibiting colon shortening.

Colon biopsy samples taken from patients with ulcerative colitis revealed the presence of neutrophilic infiltration in the colonic tissue. Additionally, erosions, ulcerations, or granulation tissue were observed, and the mucosal damage led to a reduction in the secretion of acidic mucus [12]. In the present study, we also found mucosal damage in the colon of mice with advanced ulcerative colitis, with increased inflammatory cells spreading into the submucosa and barrier breakdown (**Figure 3B** and **C**). In contrast, in the colon tissue of mice treated with curcumin, inflammatory cell infiltration and mucosal damage were inhibited (**Figure 3D** and **E**). These results may suggest that curcumin has the potential to treat UC as it inhibits inflammation and prevents mucosal damage.

It has been reported that an HFD promotes UC by inducing oxidative stress in the colon [13]. This study confirms the link between HFD and UC. Mice fed an HFD had dysplastic conduit structures in the colonic mucosa and reduced acidic mucus in the mucosal layer (**Figure 3B** and **C**). These results suggest that UC is more severe in obese individuals. In addition, an HFD leads to the formation of adipose tissue in the liver, which induces inflammation and increases adipocytes in abdominal fat [14]. It has been reported that high-fat diets exacerbate colitis severity by altering the miRNA

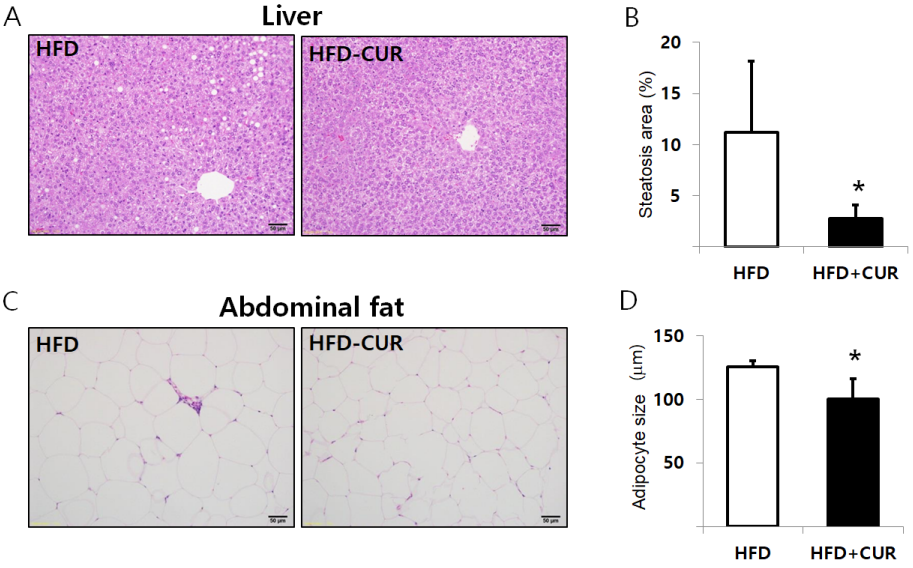

Figure 4. Effect of curcumin on steatosis and adipocyte size in the liver and abdominal adipose tissues from mice fed the HFD and HFD + CUR with DSS-induced colitis for 5 weeks. (A) Liver tissues; (B) Steatosis area in liver tissue; (C) abdominal fat tissue; (D) adipocyte size in abdominal fat tissue. Each value is presented as the mean ± standard deviation of the mean. *$P < 0.05$ vs. the HFD group, $n = 16$.

profile of visceral fat exosomes, shifting the exosomes from an anti-inflammatory to a pro-inflammatory phenotype [15]. Thus, there is a strong association between obesity and ulcerative colitis. In the present study, curcumin significantly reduced liver steatosis and adipocytes in abdominal fat (**Figure 4**), suggesting that curcumin may inhibit the progression of UC exacerbated by obesity.

Taken together, these results suggest that curcumin has anti-inflammatory properties to inhibit the progression of UC, as DSS-induced colitis in mice fed a high-fat diet resulted in histologically significant remission of colitis in the HFD + CUR group compared to the HFD group. However, further studies are needed to clarify the mechanism by which curcumin inhibits the progression of colitis. In addition, there was a significant decrease in the size of liver steatosis and abdominal fat cells in the HFD + CUR group, suggesting that curcumin not only inhibits steatosis and obesity but also inhibits the progression of colitis caused by obesity, thereby delaying the progression to cancer.

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Disclosure statement

The authors declare no conflict of interest.

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