Vitamin C Enema Advances Induction of Remission in the Dextran Sodium Sulfate-Induced Colitis Model in Rats

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Summary The current main treatment for ulcerative colitis (UC) is induction therapy by long-term administration of 5-aminosalicylic acid (5-ASA), but various side effects have been reported. Therefore, a radical cure for UC is desired. A vitamin C (VC) has anti-inflammatory effects. Therefore, this study investigated whether a VC solution enema shortens induction of remission in colitis model rats. Wistar rats (6 wk old/male) were allowed to freely ingest a 1% dextran sulfate sodium (DSS) solution for 10 d and then switched to tap water for normal breeding for 10 d (UC group). At the time of switching to tap water, an enema was performed with a 5-ASA solution (40 mg/kg/d) or VC solution (460 mg/kg/d) for 10 d. The neutrophil number, COX-2, which is an index of inflammation, and type III collagen, which is an early healing marker, were significantly increased in the UC group. However, the VC group showed decreases compared with UC groups. Furthermore, compared with UC and 5-ASA groups, the VC group showed increased expression of type I collagen, which is expressed late in healing, and significant epithelial regeneration was observed in colon tissue. The VC solution enema shortened the induction of remission by directly suppressing inflammation of damaged large intestinal tissues and promoting mucosal healing.

Key Words ascorbic acid, ulcerative colitis, inflammatory bowel disease, induction of remission, mucosal healing

Ulcerative colitis (UC) is classified as an inflammatory bowel disease (IBD) and is an inflammatory disease of unknown cause, which mainly affects colonic mucosa and forms erosions and ulcers (1). Additionally, it is designated as a specific disease similar to Crohn's disease (2). The ages of UC onset are 20–24 y for men and 25–29 y for women (2). The number of UC patients exceeds about 11 million worldwide, and it is reported that the incidence is high mainly in developed countries (2). Despite significant advances in UC research, the number of patients is still growing rapidly, and the mechanisms of onset and progression are not fully understood (2). Injuries of UC begin in the rectum, which spread continuously throughout the large intestines (3). UC is a disease that recurs and remits repeatedly with intestinal (toxic macro-colon and perforation) and extra-intestinal (primary sclerosing cholangitis, joint pain, and gangrenous pyoderma) complications (3). The risk of colorectal cancer increases when the colon is extensively affected over a long period of time (1). Symptoms can include diarrhea, mucous stool, abdominal pain, and fever, which will depend on the

extent and severity of the lesion (1).

The current UC treatments are drug therapy and blood cell removal therapy (4). It is important to achieve remission induction with a combination of treatments and maintain remission as much as possible without administration of corticosteroids or surgery with strong side effects. The drug used primarily in UC patients for induction of remission is “Pentasa,” the main component of which is 5-aminosalicylic acid (5-ASA). The mechanism of UC improvement by 5-ASA is inhibition of the rate-limiting enzyme in the synthesis of 5-lipoxygenase (5-LO) and the inflammatory substance leukotriene B₄ (LTB₄) (4). LTB₄ is present at higher concentrations in UC patients than in healthy subjects, and 5-ASA exerts anti-inflammatory effects on colonic mucosa by inhibiting the rate-limiting enzymes involved in synthesis of 5-LO and LTB₄ (4). However, side effects have been reported, such as abdominal pain, nausea, diarrhea, and rash, and long-term use of 5-ASA preparations causes severe symptoms such as hepatitis, lung disorders, blood disorders, and nephritis (5). Current treatments for UC are primarily aimed at reducing intestinal inflammation rather than healing mucosal ulcers, and mucosal healing is not regarded as a therapeutic goal in IBD treatment (6, 7). Recently, however, muco-

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sal healing was found to be a reliable indicator of therapeutic efficacy and has been suggested to be an important long-term prognostic marker (7). Therefore, to achieve good therapeutic results in the treatment of UC, treatment for both inflammation and mucosal healing of the colonic mucosa is important and necessary. Thus, the development of new therapeutic agents for UC is anticipated.

Vitamin C (VC) has a direct ameliorating effect on inflammation (8). However, humans do not express L-gluconolactone oxidase, an important enzyme in the biosynthetic pathway of VC. Therefore, humans cannot biosynthesize VC, even though VC is an essential nutrient for humans (8, 9). VC has a characteristic chemical structure of an enediol group ($\text{HO-C}=\text{C-OH}$), which is a free radical such as a superoxide anion radical (O_2^-) and hydroxyl radical ($-\text{OH}$), and the function of an electron donor to supply electrons to radicals (9). By this action, VC has a strong antioxidant activity such as protecting important biomolecules, including proteins, lipids, and nucleic acids, from damage by oxidants. Additionally, VC is closely related to immune functions and suppresses inflammation by improving the regulation of inflammatory cells, such as neutrophils and macrophages, and inflammatory mediators such as cytokines (8). VC also promotes the proliferation and migration of fibroblasts, which are essential for tissue remodeling and wound healing (8, 10). Additionally, VC increases the expression of collagen genes in fibroblasts and acts on the early synthesis of collagen (9, 11).

In this study, to examine the efficacy of VC for UC treatment, we established a rat model for induction of UC remission and investigated the effect of shortening remission.

MATERIALS AND METHODS

Animals. Six-week-old male Wistar rats were provided by Japan SLC, Inc. All rats were individually housed in plastic cages at $25 \pm 2^\circ\text{C}$. with 55% humidity in a 12-h light-dark cycle. Food and water were provided ad libitum for 1 wk after the start of breeding. This study was conducted with the approval of Kindai University Animal Experiment Committee (approval number: KAAG-2020-018).

UC exposure and remission induction in rats. Rats were randomly divided into four groups: Control ($n=6$), UC ($n=6$), 5-ASA ($n=6$), and VC ($n=6$) groups. Control group: Tap water was freely available during the experiment. UC group: UC exposure was performed by free access to a 1% dextran sulfate sodium (DSS) solution dissolved in tap water for 10 d. At the end of the DSS solution free intake period, the rats were switched to free intake of tap water and continuously reared for 10 d. 5-ASA group: An enema of a 5-ASA solution (40 mg/kg) was performed for 10 d at the same time as switching from the DSS solution to tap water. VC group: An enema of a VC solution (460 mg/kg) was performed for 10 d at the same time as switching from the DSS solution to tap water. This was calculated to have the same molar concentration (0.26 mol/L) as the 5-ASA

formulation used in patients with ulcerative colitis (12) at 46 mg/kg VC. Next, a 10-fold concentration of the VC solution was used for this experiment. The colon was excised the day after the end of the enema to measure the mRNA and protein expression levels of markers of inflammation and healing and to perform histological evaluation.

Histological analysis. Excised colon sections were fixed in 4% paraformaldehyde and stained with hematoxylin and eosin (HE) for histological analysis. Slides were stained for HE and staining patterns were interpreted by two staff pathologists. Eosinophils and neutrophils were counted in HE-stained sections to evaluate inflammation. To evaluate healing, fibroblast counting and regenerative epithelial length measurement were performed under an optical microscope at magnifications of $\times 20$ or $\times 40$. For histological evaluation, any 5 perspectives were selected from one section.

Western blotting. Protein was extracted from colon tissue by homogenization. After blocking, the primary antibody reaction was carried out overnight at 4°C . A rabbit monoclonal anti-COX-2 antibody (1 : 1,000, Abcam) was used as an indicator of inflammation. A rabbit monoclonal anti-collagen type (CT) III antibody (1 : 50,000, Abcam), which is observed early in wound healing, was used as an early indicator of healing. A rabbit monoclonal anti-CT I antibody (1 : 7,500, Abcam), which is observed late in wound healing, was used as an indicator of healing completion. A mouse monoclonal anti-GAPDH antibody (1 : 10,000, Institute for Medical Biology) was used as an internal standard protein. Secondary antibodies were rabbit IgG H & L (1 : 10,000, Institute of Medical Biology) and mouse IgG H & L (1 : 10,000, Institute of Medical Biology). These primary and secondary antibodies were diluted in 5% dry skim milk. Chemiluminescence was performed using Amersham™ ECL™ Prime (GE Healthcare Life Sciences). Bands were photographed and emission intensity was measured using an Amersham Imager 680 (GE Healthcare Life Sciences).

Reverse Transcription-Polymerase Chain Reaction (RT-PCR). mRNA was extracted from colon tissue using ISOGEN (Japan Gene), and absorbances at 260 nm/280 nm were measured by a Multiskan FC (Thermo Scientific) to calculate the mRNA concentration. Then, the mRNA concentration was adjusted to 100 ng/ μL with distilled water. Using the extracted mRNA, gene expression of trefoil factor 3 (TFF-3), which is an index of wound healing, and transforming growth factor β (TGF- β), which is a collagen synthesis-promoting factor, was examined. Glyceraldehyde-3-phosphate dehydrogenase (G3PDH) was used as an internal standard. The sequence of each primer is shown in Table 1.

A 0.5% agarose gel was prepared and electrophoresis was conducted at 100 V for 40 min in a submarine electrophoresis tank (Advance Co., Ltd.). After electrophoresis, the bands were photographed and mRNA was quantified with the Amersham Imager 680.

Statistical analysis. Measurements were calculated as the mean $\pm$ standard error. Comparisons among mul-

Table 1. The base sequence of each primer.

Gene	Base sequence	bp	Cycle
G3PDH	sense antisense	TGAAGGTCGGTGTCAACGGATTTGGC CATGTAGGCCATGAGGTCCACCAC	983 25
TFF-3	sense antisense	ATGGAGACCAGAGCCTTCTG ACAGCCTTGTGCTGACTGTA	403 25
TGF- β	sense antisense	CTACTACGCCAAAGAAGTCACC GTTGACTTGAATCTCTGCAGGC	158 30

Fig. 1. Treatment with enema of vitamin C reduces clinical symptom levels in rat colitis. Changes in body weight (A). Length of the imaged large intestines (B, C). Significant colonic atrophy was observed in the UC group compared with the control group, which indicated colonic atrophy due to DSS ingestion. However, colonic atrophy was improved in 5-ASA and VC groups compared with the UC group. Moreover, in the VC group, the colon was elongated to the same extent as that in the control group. Results are shown as the mean $\pm$ SE. * $p < 0.05$; $n = 6$. UC: ulcerative colitis, DSS: dextran sodium sulfate, 5-ASA: 5-aminosalicylic acid, VC: vitamin C.

tiple groups were made using a one-way ANOVA with Tukey Kramer's test; $p < 0.05$ was considered as significant.

RESULTS

Treatment with enema of vitamin C reduces clinical symptom levels in rat colitis

Figure 1 shows the changes in body weight during the experiment and the colon and intestine at the time of dissection. The control group showed normal weight gain (Fig. 1A). Weight loss was also observed on days 12 and 13 in the other three groups exposed to UC by DSS. Switching to tap water was found to improve weight loss. Furthermore, enemas of 5-ASA or VC solutions improved the weight loss due to DSS exposure, and the enema of the VC solution recovered body weight to close to that of the control group. The length of the

imaged large intestines is shown in Fig. 1B. Significant colonic atrophy was observed in the UC group compared with the control group (Fig. 1C, $p < 0.05$), which indicated colonic atrophy due to DSS ingestion. However, colonic atrophy was improved in 5-ASA and VC groups compared with the UC group. Moreover, in the VC group, the colon was elongated to the same extent as that in the control group.

Vitamin C solution enema reduces inflammation in rat colitis and improves histologically

A colon histological image with HE staining of each group is shown in Fig. 2A, B, C, D. Crypt structures, the normal structure of the large intestines, were observed in tissues of the control group (Fig. 2A). However, inflammatory cells, such as eosinophils and neutrophils, and fibroblasts involved in collagen synthesis were not observed. However, in tissues of UC (Fig. 2B), 5-ASA

Fig. 2. Vitamin C solution enema reduces inflammation in rat colitis and improves histologically. Colonic tissues were stained with hematoxylin and eosin. (A) Control group; (B) UC group; (C) 5-ASA group; (D) VC group. Scale bar: 50 μ m. (E) neutrophil counts, (F) eosinophil counts, and (G) fibroblast counts were counted by two pathologists from the rectal section of each group. Western blotting of COX-2 and GAPDH (internal standard) protein expression levels in colon tissue (H). Results are shown as the mean $\pm$ SE. * p <0.05, ** p <0.01; n =6. UC: ulcerative colitis, 5-ASA: 5-aminosalicylic acid, VC: vitamin C.

(Fig. 2C), and VC (Fig. 2D) groups, loss of the crypt structure and many inflammatory cells were observed. The UC group had a large number of fibroblasts, and 5-ASA and VC groups had a small number of fibroblasts. The numbers of neutrophils that had migrated because of inflammation was counted in HE-stained images of the colon tissue (Fig. 2E). A significant increase in the number of neutrophils was observed in UC, 5-ASA, and VC groups that received DSS compared with the control group (p <0.05). Moreover, the numbers of neutrophils in 5-ASA and VC groups were reduced significantly compared with that in the UC group (p <0.05). Furthermore, the neutrophil count was reduced in the VC group compared with the 5-ASA group. Figure 2F shows the numbers of eosinophils that migrated because of inflammation, which were calculated from HE-stained images of the large intestines. The number of eosinophils was significantly increased in UC, 5-ASA, and VC groups that received DSS (p <0.05). Moreover, the number of eosinophils was significantly reduced in the VC group compared with the UC group (p <0.05). Furthermore, the number of eosinophils tended to decrease in the VC group compared with the 5-ASA group. In HE-stained images of colon tissues, the numbers of fibroblasts that proliferated and differentiated during inflammation and were involved in collagen synthesis were counted (Fig. 2G). A significant

increase in the number of fibroblasts was observed in UC, 5-ASA, and VC groups compared with the control group (p <0.05). The 5-ASA group showed a tendency for a decrease in the number of fibroblasts compared with the UC group. Additionally, the VC group showed a significant reduction in the fibroblast number compared with UC and 5-ASA groups (p <0.05). The amount of COX-2 protein produced by neutrophils that migrated because of inflammation and used as an indicator of inflammation was quantified by western blotting (Fig. 2H). The UC group showed a significant increase in COX-2 protein expression compared with the control group (p <0.01). Additionally, 5-ASA and VC groups showed a tendency for a lower expression level of COX-2 protein compared with the UC group. Furthermore, the VC group showed a tendency for a decrease in the COX-2 protein expression level compared with the 5-ASA group.

Enema of vitamin C solution activates tissue repair and promotes healing of damaged colonic mucosa

The amount of CT III protein expressed during the early stages of mucosal healing was quantified by western blotting (Fig. 3A). The UC group showed an increasing tendency of CT III protein expression compared with the control group. Furthermore, the 5-ASA group showed a downward trend in CT III protein expression compared with the UC group. The VC group showed a

Fig. 3. Enema of vitamin C solution to damaged colon tissue activates collagen synthesis and promotes tissue repair. Western blotting of CT III (A) or CT I (B) and GAPDH (internal standard) protein expression levels in colon tissue. RT-PCR analyses of TGF- β (C) or TFF-3 (D) and G3PDH (internal standard) mRNA expression levels in colon tissue. Results are shown as the mean $\pm$ SE. * p <0.05, ** p <0.01; n =6. UC: ulcerative colitis, 5-ASA: 5-aminosalicylic acid, VC: vitamin C.

Fig. 4. Enema of vitamin C solution promoted tissue repair of damaged colon tissue and accelerated induction of remission. Colonic tissues were stained with hematoxylin and eosin. The line indicates the regenerated epithelium. (A) Normal control group; (B) UC group; (C) 5-ASA group; (D) VC group. Scale bar: 100 μ m. (E) Regenerated epithelial length confirmed in 3 groups (UC, 5-ASA, VC) ingesting DSS. Results are shown as the mean $\pm$ SE. ** p <0.01, *** p <0.001; n =6. UC: ulcerative colitis, 5-ASA: 5-aminosalicylic acid, VC: vitamin C.

significant decrease in the CT III protein expression level compared with the UC group (p <0.05) and a decreasing tendency compared with the 5-ASA group. Expression of CT I protein during wound healing was quantified by western blotting (Fig. 3B). The 5-ASA group showed an increasing trend in CT I protein expression compared with the UC group. The VC group showed a significant increase in CT I protein expression compared with the UC group (p <0.05) and an increasing tendency of CT I protein expression compared with the 5-ASA group. The mRNA expression level of TGF- β , which is a factor that promote differentiation from CT III to CT I, was quantified by RT-PCR (Fig. 3C). The

5-ASA group showed an increasing tendency of TGF- β mRNA expression compared with the UC group. Additionally, the VC group showed a significant increase in the TGF- β mRNA expression level compared with the UC group (p <0.05) and an increasing tendency of TGF- β mRNA expression compared with the 5-ASA group. In the injured mucosal epithelium, the mRNA expression levels of TFF-3, which is an epithelial repair-promoting factor and mucosal protective factor, were quantified by RT-PCR (Fig. 3D). The UC group showed a decreasing tendency the TFF-3 mRNA expression compared with the control group. However, the 5-ASA group showed a tendency of increased TFF-3 mRNA

expression compared with the UC group. Furthermore, the VC group had significantly increased expression of TFF-3 mRNA compared with the UC group ($p < 0.05$) and showed an increasing tendency compared with the 5-ASA group, and the expression was almost the same as that in the control group. In colon histology images of HE staining, normal mucosal epithelium was observed in the control group (Fig. 4A). In the UC (Fig. 4B), 5-ASA (Fig. 4C), and VC (Fig. 4D) group, epithelial regeneration from damaged colon tissue was observed, and enemas of 5-ASA or VC solutions caused further expansion of the regenerated epithelium. The results of measuring the length of the regenerated epithelium in stained image of the large intestinal tissue are shown in Fig. 4E. 5-ASA and VC groups showed significant elongation of the regenerated epithelium compared with the UC group ($p < 0.01$).

DISCUSSION

Studies have reported the therapeutic effect of intraperitoneal VC administration on ulcerative colitis in mice (13, 14). It was important that the amount of vitamin C that acted on inflamed colon tissue directly could be less than the dose of vitamin C administered orally or intravenously. Therefore, similar to 5-ASA, it was considered important to deliver vitamin C directly to inflamed colon tissue for antioxidant, anti-inflammatory, and repair effects in the affected area. However, the direct effects of VC enemas on ulcerative colitis had not been studied. In this study, we established a rat model of UC remission and investigated whether a VC enema shortened the induction of remission. The method to prepare the UC remission-induced rat model in this study was as follows. Similar to a previous study, the model was established by allowing a 1% DSS solution to be freely ingested for 10 d (days 1–10) and then switching to tap water and allowing normal breeding for 10 d (days 11–20).

During the experimental period, weight gain was observed in the control group and weight loss was observed on days 12 and 13 in UC, 5-ASA, and VC groups exposed to UC by DSS.

At the end of the experiment, colonic atrophy due to ingestion of DSS was observed in the UC group compared with the control group. Although the mechanism of DSS-induced UC has not been clarified, it has been reported that DSS damages colonic epithelial cells, which causes inflammatory cells to infiltrate the damaged site and cause inflammation (15). Additionally, by switching from DSS to free intake of tap water, body weight was eventually improved. In addition, the enemas of 5-ASA and VC solutions further improved weight loss due to DSS exposure. In particular, the VC solution enema restored body weight to the same level as the control group. The enemas of 5-ASA and VC solutions also improved colonic atrophy caused by DSS ingestion.

Next, HE-stained colon sections were used to count inflammatory cells. After counting the number of neutrophils migrating because of inflammation, UC, 5-ASA,

and VC groups, which were administered DSS, showed significant increases in the numbers of neutrophils compared with the control group. Damage to colonic epithelial cells by DSS causes necrosis, and intestinal bacteria invade the submucosa (15, 16). Bacteria and necrotic cells that invade the submucosa are phagocytosed by intestinal macrophages and release CXCL8 (16). CXCL8 activates neutrophils that migrate to the injury site and attach to the vascular endothelium, which causes inflammation (17). Furthermore, CXCL8 is more highly expressed in activated macrophages and colon epithelial cells of the colon epithelium in UC patients than in the normal colon epithelium (17, 18). Migrating neutrophils detoxify bacteria by releasing ROS and anti-bacterial proteins (18). Neutrophils eventually undergo apoptosis and are removed by macrophages, thereby eliminating the inflammatory response (18). However, delayed healing of an injured site has been reported to cause neutrophils to lodge and continue the inflammatory response, which further impedes wound healing and causes necrosis (17, 18). In this study, a significant decrease in the number of neutrophils was observed in the VC group compared with UC and 5-ASA groups. These results suggested that the VC solution enema in the rat model with UC remission suppressed the release of activated macrophage-derived CXCL8 as well as the migration and activation of neutrophils, which induced apoptosis and rapidly reduced the number of neutrophils. ROS released from neutrophils oxidizes VC itself, suppresses lipid peroxidation of active oxygen, removes ROS involved in the inflammatory reaction, and further suppresses the inflammatory reaction. Because the number of inflammatory cells in the VC group was lower than that in the UC group, the degree of destruction of crypt structures in the colon epithelium was reduced.

The UC group showed a significant increase in COX-2 protein expression compared with the control group. When inflammation is caused by exposure to DSS, inflammatory cytokines such as TNF- α are released and COX-2 is expressed by macrophages, neutrophils, and fibroblasts, which causes pain and produces prostaglandin E2 (PGE2) (19). Inhibition of COX-2 reduces pain and inflammation (19). Therefore, in this study, the enema of the VC solution reduced inflammatory cells, such as neutrophils and eosinophils, and the expression level of the COX-2 protein compared with the UC group.

When colonic epithelial cells are injured by DSS, intestinal bacteria enter, inflammation is induced, and inflammatory cells, such as eosinophils and neutrophils, migrate, activate, and express COX-2. Neutrophils induce tissue damage and persistent inflammation by ROS generation, and COX-2 induces pain by PGE2 induction. However, the VC solution enema suppressed the inflammatory response by reducing eosinophils and neutrophils that had migrated because of DSS exposure and suppressing the expression of COX-2. VC also removed ROS released from neutrophils, which further suppressed tissue damage and persistent inflammation, and accelerated the transition of UC to remission.

Mucosal healing was examined to assess maintenance of remission. Mucosal healing has not been considered as a therapeutic goal for IBD (6, 7). However, mucosal healing is a reliable indicator of therapeutic efficacy and has been suggested to be an important long-term prognostic marker (7). In this study, the UC group showed a significant increase in the fibroblast number compared with the control group. When colon epithelial cells are damaged by DSS and form an ulcer, repair of the damaged area begins (19). The damaged area is repaired by collagen synthesized from fibroblasts.

Fibroblasts are transferred to the lesion by the actions of platelet-derived growth factor, fibroblast growth factor-2 (FGF-2), and TGF- β produced by intestinal macrophages (19). It has been reported that these actions activate fibroblasts and induce their proliferation (19). Activated fibroblasts synthesize collagen for tissue repair and scar formation in damaged areas of colonic tissue (19). In this study, the VC group showed a significant reduction in the fibroblast number compared with UC and 5-ASA groups. It has been reported that VC acts on fibroblasts and as a cofactor for lysyl and prolyl hydroxylases, which promotes collagen hydroxylation and synthesis (20, 21). Therefore, the site of tissue damage was reduced and the number of migrating fibroblasts was reduced by suppressing the inflammation caused by the enema of the VC solution. Furthermore, VC activated fibroblast proliferation and differentiation, promoted collagen synthesis, and further reduced the number of fibroblasts.

We measured the level of CT III protein expressed in the early stage of wound healing and that of CT I protein expressed after differentiation and healing with CT III. As a result, the UC group showed an increase in the expression of CT III protein with compared with the control group. The VC group showed a significant reduction in CT III protein expression compared with the UC group. However, the UC group showed a decrease in CT I protein expression compared with the control group, and the VC group showed a significant increase in CT I protein expression compared with the UC group. CT III is observed in the early stages of wound healing and is a crucial regulator of CT I formation, an indicator of scar formation (22). Additionally, CT III is converted to CT I during the healing process (22). In this study, CT III protein expression levels were lower and CT I protein expression levels were higher in the VC group compared with the UC group. This suggests that the VC solution enema after DSS exposure promoted collagen synthesis in damaged colon tissue, which replaced CT III with CT I and promoted scar formation.

Additionally, the VC group showed an increase in TGF- β mRNA expression compared with UC and 5-ASA groups. TGF- β acts on fibroblasts, promotes CT I production, and plays a major role in tissue repair (23). This also supports our opinion that the VC solution enema promoted scar formation in damaged colon tissue.

In the UC group, the mRNA expression level of TFF-3, an epithelial repair-promoting factor and mucosal pro-

TECTIVE factor, was lower in the injured mucosal epithelium than in the control group. Additionally, the mRNA expression level of TFF-3 was significantly increased in the VC group compared with the UC group, and the expression level was restored to the same level as that in the control group. TFF-3 is distributed in all gastrointestinal tracts, which promotes mucosal protection, regeneration of damaged mucosa, and gastrointestinal wound healing (24, 25). In the UC group, conversion from CT III to CT I did not function well because the inflammation continued and mucosa-protective TFF-3 was decreased even after switching to tap water. However, infusion of the VC solution rapidly suppressed inflammation, promoted scar formation, and restored the expression level of TFF-3.

Finally, HE-stained sections were used to observe colon tissue repair. Compared with the UC group, the VC group showed more tissue repair at many sites, significant elongation of the regenerated epithelium, and the final stage of the healing process. After sufficient scar formation by fibroblasts, FGF-2 produced by intestinal macrophages stimulates the migration of epithelial cells and initiates formation of the regenerating epithelium overlying damaged tissue (19). In this study, the VC group showed significant elongation of the regenerating epithelium compared with the UC group. Therefore, the enema of the VC solution promoted mucosal healing and migration of epithelial cells, and rapidly formed a regenerated epithelium in damaged colon tissue.

Also, it has been reported that rats have the ability to biosynthesize VC (8). However, in this study, the UC group did not show a significant improvement in morbidity compared with the control group. Thus, it is considered difficult to suppress the inflammation of ulcerative colitis with biosynthetic VC alone.

These results suggest that the VC solution enema promotes more mucosal healing, shortens the induction period of remission, and maintains remission faster than 5-ASA, which is a basic therapeutic agent for UC.

Authorship

Research conception and design: TH, KT, MK, and TI; experiments and statistical analysis of the data: TH, KT, and MK; writing of the manuscript: TH and TI. All authors read and approved the final manuscript.

Disclosure of state of COI

The authors declare that they have no conflict of interests.

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