A New Role for Cathelicidin in Ulcerative Colitis in Mice

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Cathelicidin, an antimicrobial peptide of the innate immune system, modulates microbial growth, wound healing, and inflammation. However, its association with inflammatory bowel diseases (IBDs) is unknown. Our objective was to determine whether cathelicidin would exert a modulatory effect on the progression of IBD and, if so, investigate the mechanism of action through which this effect occurred. We evaluated the potential for a synthetic cathelicidin, the mouse cathelin-related antimicrobial peptide (mCRAMP), to prevent the initiation and promote the healing of lesions from inflammatory colitis that was experimentally induced in mice with dextran sulfate sodium (DSS). During the experiment, mCRAMP was given: (i) as a parallel treatment starting together with 3% DSS feeding, and (ii) as a posttreatment starting 7 days after 3% DSS feeding. The body weight, fecal microflora populations, clinical symptoms, and histologic findings of colonic tissues were measured. Relative gene expression of mucins (*MUC1, MUC2, MUC3,* and *MUC4*) in colonic tissues was determined by real-time polymerase chain reaction. Intrarectal administration of mCRAMP ameliorated DSS-induced colitis with negligible effects on mucosal healing. The peptide also significantly reduced the increased number of fecal microflora in colitis animals. It reversed the decline of colonic mucus thickness during colitis through upregulation of the expression of mucin genes. Treatment with mCRAMP also prevented colitis development by suppressing the induction of apoptosis by DSS. The current study demonstrates for the first time that intrarectal administration of cathelicidin may be a novel therapeutic option for IBDs. Exp Biol Med 232:799–808, 2007

Key words: mCRAMP; antimicrobial peptide; IBD; mucus

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Introduction

Inflammatory bowel diseases (IBDs), including ulcerative colitis (UC) and Crohn disease (CD), are disorders of the gastrointestinal tract that are characterized by chronic, relapsing inflammation. Despite the unclear etiology, it has been reported that immunologic abnormality and enteric microflora are associated with the pathogenesis of the diseases (1, 2). Resistance of colitis development in germ-free animals and inflammation induced by reintroduction of intestinal flora in these animals have indicated a critical role for microflora (3). Overload of microflora has been proposed to provoke abnormal immune responses in the colon and result in inflammation (4). The therapeutic effects of tumor necrosis factor-α (TNF-α)–neutralizing antibody (5) and interleukin-10 (IL-10) administration (6) furthermore showed that one of the major goals of IBD treatment is to control the dysregulated immune response in the colon and break the proinflammatory cascade cycle leading to tissue necrosis. In fact, several immunomodulatory agents, such as corticosteroids, 6-mercaptopurine, and azathioprine, have been commonly used for human IBDs. Nevertheless, some patients are refractory to these drugs, and some may even have adverse side effects, like osteoporosis and cataracts, after prolonged usage (7). The current IBD treatment mainly focuses on alleviating the inflammatory response by bringing the disease into remission rather than preventing the relapse of the disease. More importantly, persistent inflammation in the colon may increase the risk of colorectal cancer (8). Therefore, a safer and more efficient therapy is needed for the treatment and prophylaxis for IBDs, and perhaps also for colorectal cancer in humans.

The colonic mucus layer has been suggested as another potential therapeutic target for IBD. This is based on the histologic findings that IBD patients often show thinner mucus layer and depletion of goblet cells in the colonic epithelium (9). The mucus layer, which is composed of mainly mucins and trefoil peptides, acts as a physical barrier to protect the epithelium from agents disturbing epithelium integrity. It may also prevent the intestinal microflora from triggering abnormal immune responses (10). A recent study

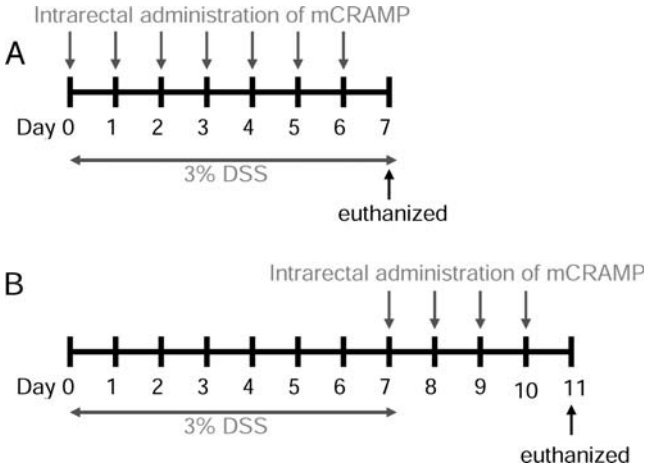

Figure 1. Experimental protocols of the study. (A) Parallel treatment: study of the preventive effects of mCRAMP, in which mCRAMP was given during the DSS administration period. (B) Posttreatment: study of the healing effects of mCRAMP, in which mCRAMP was given after DSS administration.

by Van der Sluis *et al*., in which mice with disrupted mucus synthesis suffered from more serious colitis, emphasized the importance of mucus in inflammation (11).

Cathelicidin is an endogenous antimicrobial peptide present in the surface of epithelial cells in the colon and stomach (12). It is one important effector molecule of innate immune defense. It inhibits the growth of various microorganisms, including bacteria, fungi, certain parasites, and viruses (13), by membrane permeabilization. Besides this direct action, recent studies have revealed the multiple functions of cathelicidin in many other activities related to inflammation and wound healing. Human cathelicidin has been reported to modulate the activity of immune and inflammatory cells (14, 15) and promote re-epithelialization of human skin wounds (16). Larrick *et al*. (17) have additionally shown that cathelicidin can alter inflammation by neutralizing the toxic effects of lipopolysaccharide (LPS). Its less-conserved C-terminal domain binds LPS and prohibits LPS-induced responses, such as nitric oxide release and generation of tissue factor by macrophages.

A recent study has revealed that the expression of cathelicidin is altered in patients suffering from UC but not CD (18). Immunohistochemistry showed that the expression of cathelicidin is significantly higher in both inflamed and noninflamed colon mucosa from UC patients compared with healthy subjects. This finding seems to indicate the functional role of cathelicidin in IBD. We have previously tested the influence of cathelicidin on ulcer healing in a rat model. It was discovered that cathelicidin could promote ulcer healing (19). Cathelicidin could induce proliferation of gastric epithelial cells through transforming growth factor alpha (TGF-α)–dependent transactivation of epidermal growth factor receptors and its related signaling pathway. Based on these observations, it is likely that cathelicidin also plays an essential role in the development and healing of mucosal damage and inflammation in UC.

Therefore, the present study sought to elucidate the efficacy of cathelicidin in IBD. In this regard, various concentrations of a mouse cathelin-related antimicrobial peptide (mCRAMP) were administered intrarectally to the targeted tissues with colitis induced by dextran sulfate sodium (DSS). We examined both the preventive and healing effects as well as the mechanism of action of mCRAMP on IBD, in particular to the alterations of microflora and mucosal mucus during colitis.

Materials and Methods

Animals. Male balb/c mice (6–8 weeks old) were used in the following experiments. They were allowed free access to standard laboratory chow (Ralston Purina, Chicago, IL) and tap water. All animals were housed in an air-conditioned room with controlled temperature (22°C ± 1°C), humidity (65%–70%), and day/night cycle (12:12-hr light:dark). The present study was approved by the University of Hong Kong Committee on the Use of Live Animals for Teaching and Research.

Induction of Colitis. Mice were induced with acute colitis by being given 3% DSS (molecular weight, 36–50 kDa; ICN Pharmaceuticals, Costa Mesa, CA) according to the method described by Liu *et al*. (8). The 3% DSS was given in their drinking water for 7 days (from Day 0 to Day 7). Normal control mice received tap water throughout the experiment.

mCRAMP Treatment. To evaluate the preventive and healing effects of mCRAMP on UC in mice, mCRAMP was given either as a parallel treatment, starting together with DSS feeding, or as a posttreatment, starting 7 days after 3% DSS feeding. The full length of the mature mCRAMP peptide was purchased from Innovagen (Lund, Sweden), and the peptide was dissolved in phosphate-buffered saline (PBS) for rectal administration.

Parallel Treatment. The mice were divided into two major groups: a normal group receiving tap water alone and a disease group with DSS treatment. Each group contained three subgroups ($n = 12$ in each group), which were treated with rectal administration of mCRAMP as follows: 0 (PBS control), 2.5 mg/kg per day mCRAMP, and 5.0 mg/kg per day mCRAMP. The peptide was intrarectally administrated once daily for 7 consecutive days (Fig. 1A). Mice were kept in an inverted position for 1 min after each administration to prevent leakage of the peptide from the anus. The PBS control mice received equal volumes of PBS (i.e., 100 μl) intrarectally. All mice were euthanized at Day 7 by cervical dislocation. The colonic tissues were collected and stored at –70°C for further analyses.

Posttreatment. After treatment with DSS for 7 days, mice ($n = 8$ in each group) were treated with intrarectal administration of mCRAMP (0, 2.5, and 5.0 mg/kg per day) for 4 consecutive days (Fig. 1B). The eight healthy mice

Table 1. Effects of mCRAMP Treatment Given During DSS Administration on Clinical Symptoms in Ulcerative Colitis

	Normal[a]			3% DSS[a]		
mCRAMP (mg/kg per day)	0	2.5	5	0	2.5	5
Gross bleeding (% animals)	0	0	0	92*	83	17**
Loose stools (% animals)	0	0	0	100*	100	92**
Diarrhea (% animals)	0	0	0	92*	100	34**
Weight loss (% animals)[b]	0	0	0	100*	83	92**
Disease activity index	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	3.0 ± 0.13*	2.7 ± 0.16	1.0 ± 0.16**
Crypt scores	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	3.0 ± 0.31*	2.7 ± 0.25	1.5 ± 0.29**

[a] $n = 12$ per group.
[b] Weight loss defined as a 5% drop in body weight.
* $P < 0.01$ compared with the respective normal group; ** $P < 0.05$ compared with the respective colitis group without mCRAMP treatment.

without DSS and mCRAMP administration were used as normal controls. All animals were euthanized at Day 10.

Clinical Symptoms. In all tested animals, body weight, stool consistency, and presence of gross bleeding were recorded. After colon removal at the time of animal euthanization, the length of colon from colo-cecal junction to the anal verge was measured. Disease activity index (DAI) was calculated according to the method described by Cooper *et al*. (20). In brief, body weight loss, stool consistency, and bleeding were scored from 0 to 4, and DAI is the combined score divided by 3.

Morphologic Analysis. Colonic sections from all animals were fixed in 10% formalin solution (pH 7.4). Histologic examination was performed with hematoxylin-eosin staining in paraffin sections after longitudinal sections of the colon were made. The length of crypt was measured from the base to the outermost part of the crypt under an image analyzer (Q500IW; Leica Image Systems, Cambridge, England) at ×100 magnification. Severities of colitis were graded microscopically based on the integrity of crypt: score 0, intact crypt; score 1, loss of the basal one third of the crypt; score 2, loss of the basal two thirds of the crypt; score 3, loss of entire crypt with the surface epithelium remaining intact; score 4, loss of both the entire crypt and surface epithelium (i.e., erosion; Ref. 20). All analyses were performed blindly.

Myeloperoxidase Activity (MPO) in the Colonic Tissue. Colonic MPO activity was measured as described previously (21). In brief, colon tissue was homogenized in an ice-cold 50 mmol/ml PBS (pH 6.0) solution with 0.5% hexa-decyl-trimethyl-ammonium bromide. The homogenate was freeze thawed three times, followed by repeated sonication for 60 secs each and then centrifugation for 20 mins at 14,000 rpm at 4°C. The activity of the supernatant was determined spectrophotometrically at 450 nm. The final value was expressed as enzyme units per milligram of protein.

Fecal Microflora Count. Quantitative fecal micorflora studies were performed on freshly passed stools. The stool collected was resuspended in sterile PBS. After serial dilutions were done to give final concentrations of 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7}, portions (100 μl) of each dilution were spread on brain-heart infusion agar (Sigma, St. Louis, MO) and blood agar (5% defibrinated sheep blood in Columbia agar base; Sigma) for aerobic and anaerobic microflora analysis, respectively. Plates inoculated for aerobes were incubated in air for 24 hrs at 37°C. Medium for anaerobe counting was incubated in an anaerobic chamber (80% nitrogen, 10%, hydrogen, and 10% carbon dioxide) for 48 hrs at 37°C. The total amount of microflora was represented as logarithms of the total number of colony forming units (CFUs) found per dry weight of the stool.

Histology and Immunohistochemistry. *Assessment of Mucus Thickness.* Colonic samples were fixed in formalin overnight and embedded in paraffin. Sections (5-μm) were made and stained with the periodic acid-Schiff technique as previously described by Ma *et al*. (22). The sections were counterstained with Harris hematoxylin and mounted in Permount (Fisher Scientific, Philadelphia, PA). The mucus-containing cells were stained purple-red. The thickness of the mucus layer was measured perpendicular to the mucosal surface from the edge of the epithelium to the outermost part of the mucus layer under an image analyzer (Q500IW) at ×100 magnification. Three measurements were taken per field, approximately six consecutive fields per section were measured, and results were expressed as the ratio of the thickness of the mucus layer to the thickness of the total mucosa.

Assessment of Apoptosis. The terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) method described previously was used to stain apoptotic cells (23). The number of apoptotic cells was counted in four to six randomly selected fields at ×200 magnification.

Quantitative Analysis of Mucin Gene Expression. Total RNA was isolated from mouse colonic tissues with TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. A total of 2.5 μg extracted RNA was used as the template for cDNA synthesis using the Thermoscript reverse transcription–polymerase chain reaction (RT-PCR) system (Invitrogen). Quantitative real-time PCR

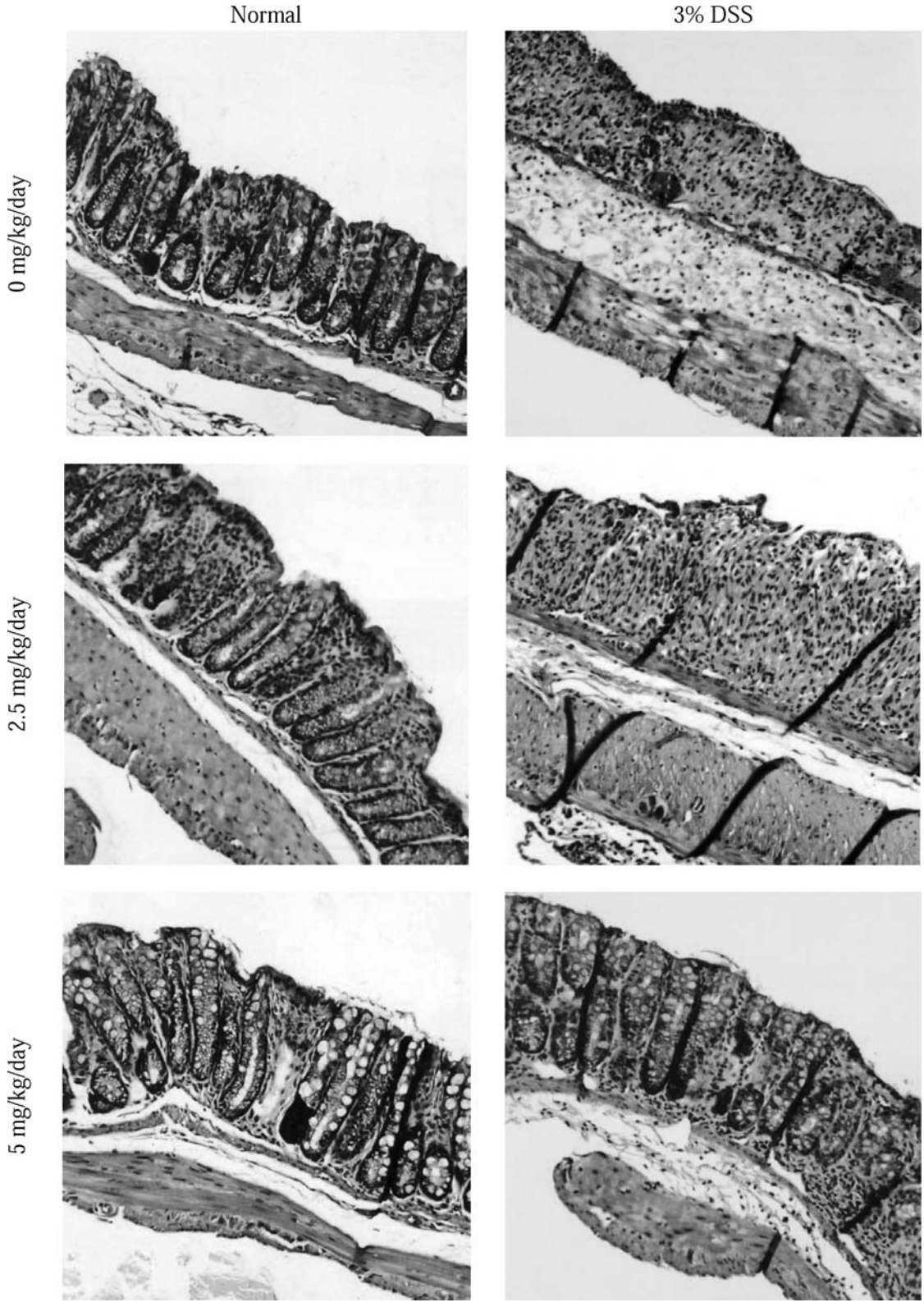

Figure 2. Representative histologic findings in mice with parallel mCRAMP treatment. Colonic tissues from mice with different mCRAMP treatments were fixed in 10% formalin and embedded in paraffin. Sections (5-μm) were made and stained with hematoxylin-eosin (original magnification ×100). DSS completely destroyed mucosal structure and decreased crypt formation. Mouse CRAMP preserved the mucosal crypt structure.

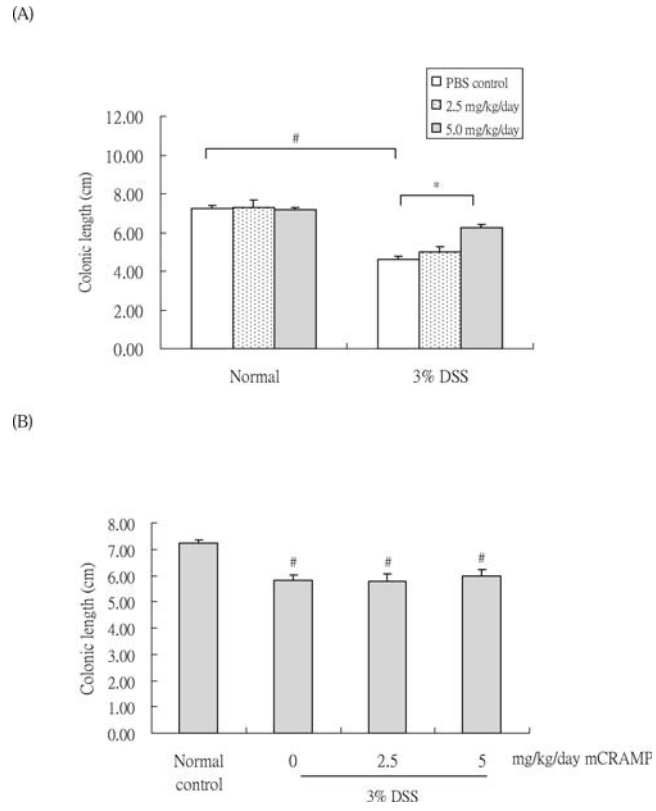

Figure 3. Effects of intrarectal administration of mCRAMP on colonic length in normal or colitis mice. (A) Parallel treatment. (B) Posttreatment. Colonic length was measured from the colo-cecal junction to the anal verge on Day 7 and Day 11, respectively. Values are mean ± SE ($n = 12$ and 8 per group in treatments A and B, respectively). #$P < 0.01$ when compared to the normal control group. *$P < 0.05$ when compared to the PBS group with DSS administration.

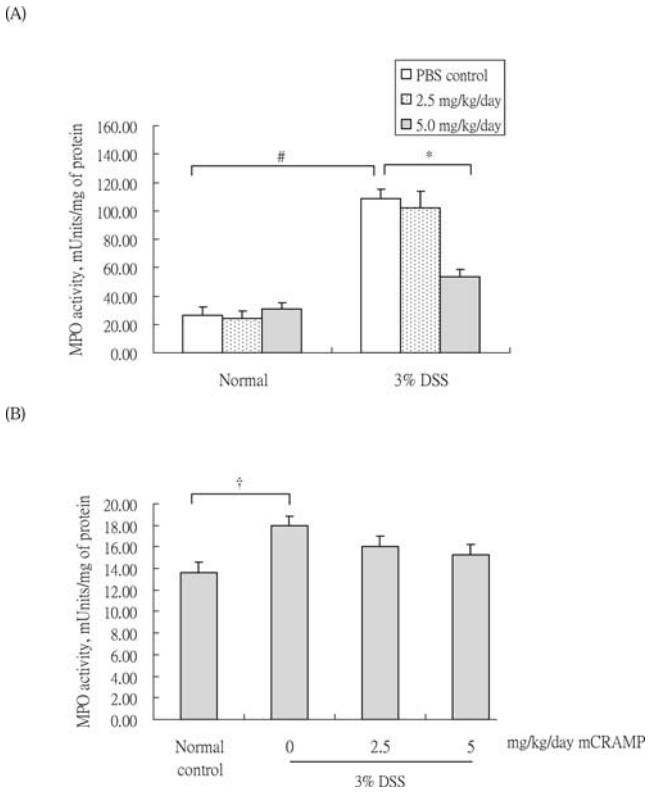

Figure 4. Effects of intrarectal administration of mCRAMP on colonic MPO activity in normal or colitis mice. (A) Parallel treatment. (B) Posttreatment. MPO activity was calculated as enzyme units and was normalized by the amount of protein. Values are mean ± SE ($n = 12$ and 8 per group in treatments A and B, respectively). †$P < 0.05$ and #$P < 0.01$ when compared to the normal control group. *$P < 0.05$ when compared to the PBS group with DSS treatment.

was performed for *MUC1*, *MUC2*, *MUC3*, *MUC4*, and β-*actin* using the following primers pairs: *MUC1*, sense primer 5′-TGGATTGTTTCTGCAGATTTT-3′ and antisense primer 5′-CCTGACCTGAACTTGATGCT-3′; *MUC2*, sense primer 5′-CCCAGAAGGGACTGTGTATG-3′ and antisense primer 5′-TGCAGACACACTGCTCACA-3′; *MUC3*, sense primer 5′-TGTTCAGCTTTACTGTGTTTCAA-3′ and antisense primer 5′-TTGCATGTCTCCTCAGGATT-3′; *MUC4*, sense primer 5′-TCATCCTCCTCAGGATTGAC-3′ and antisense primer 5′-AAATGCCCTGATCTGGTAAA-3′; β-*actin*, sense primer 5′-TCGCCATGGATGACGATA-3′ and antisense primer 5′-ATCACACCCTGGTGCCTA-3′. The cDNA was amplified using iQ SYBR Green supermix (Bio-Rad Laboratories, Hercules, CA) on the iCycler thermal cycler (Bio-Rad), programmed for 95°C for 10 mins, then 40 cycles of denaturation (95°C for 15 secs), annealing (59°C for 15 secs), and extension (72°C for 15 secs). The amplification results were detected and analyzed using the iQ5 real-time PCR detection system. The gene signals were standardized against the corresponding β-*actin* signal, and results were expressed as the ratio of each molecule to β-*actin*.

Statistical Analysis. All data were expressed as mean ± standard error (SE). Means were compared by the use of ANOVA, and a P value of <0.05 was considered to be statistically significant.

Results

Mouse CRAMP Prevented Colitis Development in Mice. *Clinical Symptoms.* To determine the effects of mCRAMP on colitis, the synthetic peptide (0, 2.5, and 5.0 mg/kg per day) was administered intrarectally to mice daily during colitis induction. The same doses of the peptide were injected to normal mice without colitis as the control group, which showed no clinical symptom. However, animals with colitis and no mCRAMP treatment showed severe clinical symptoms, such as diarrhea, gross bleeding, and higher DAI (Table 1). Interestingly, administration of mCRAMP at the dose of 5.0 mg/kg per day significantly ameliorated the disease severity, whereas no obvious influence was found at the dose of 2.5 mg/kg per day.

Colonic Length. The colonic length in colitis mice without mCRAMP treatment was significantly shorter than in the normal mice (Fig. 3A). Mice that received treatment with mCRAMP at the higher dose, 5.0 mg/kg per day, had a significantly longer colonic length. However, no significant

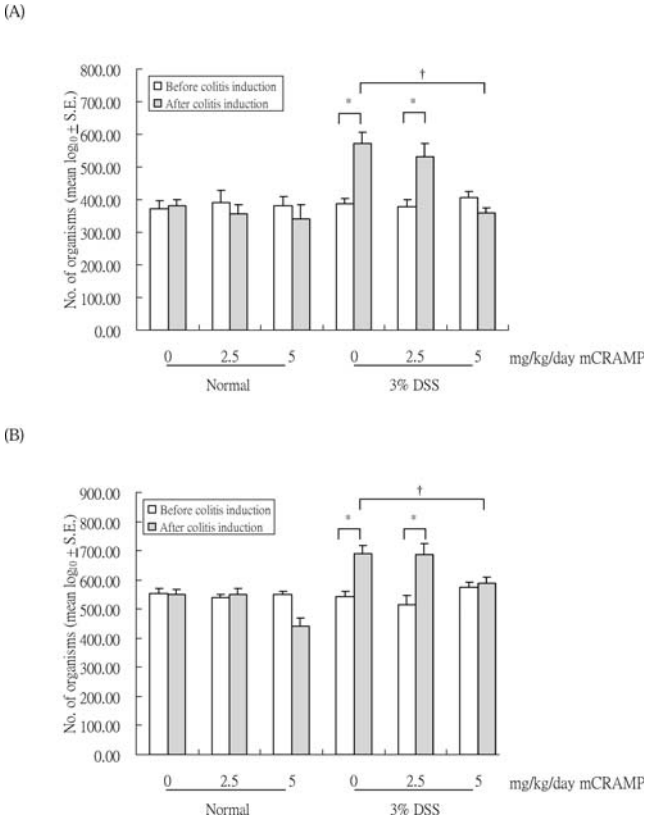

Figure 5. Effects of intrarectal administration of mCRAMP on fecal microflora populations before and after colitis induction in mice with parallel DSS treatment. (A) Aerobic microflora. (B) Anaerobic microflora. The quantification was checked before and after colitis induction (i.e., on Day 0 and Day 10, respectively) in freshly passed stools. Amount of microflora was calculated as $\log_{10}$ of the total number of CFUs found per dry weight (gram) of feces. Values are mean ± SE ($n = 12$ in each group). *$P < 0.05$ compared with the respective group before colitis induction. †$P < 0.05$ compared with the respective group with no mCRAMP treatment.

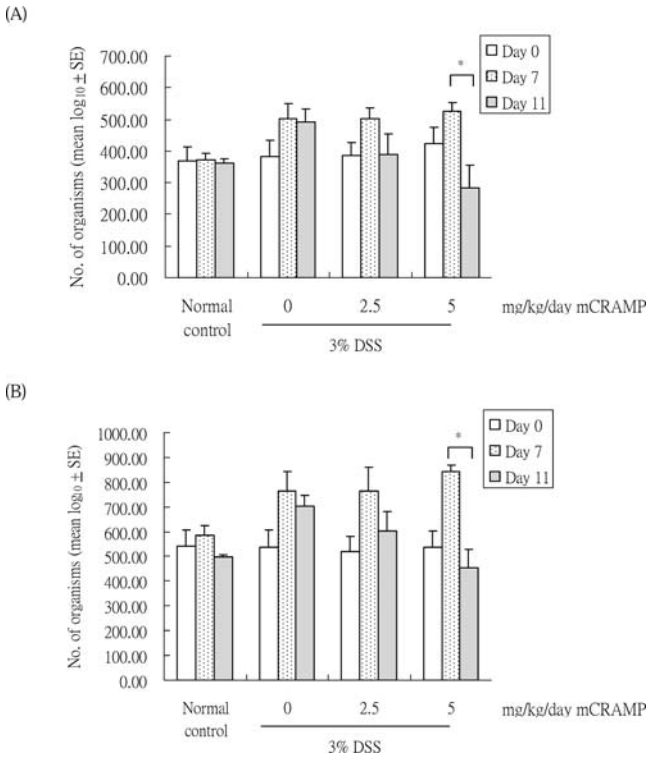

Figure 6. Effects of intrarectal administration of mCRAMP on fecal microflora populations in mice with DSS and post-mCRAMP treatment. (A) Aerobic microflora. (B) Anaerobic microflora. The quantification was performed before and after colitis induction and 4 days after mCRAMP treatment (i.e., Day 0, Day 7, and Day 11). The amount of microflora was calculated as in the parallel treatment. Values are mean ± SE ($n = 8$ in each group). *$P < 0.05$ compared between groups with colitis and 4 days after stopping colitis induction (i.e., stopped giving DSS administration).

change was observed in colitis animals given the lower dose of mCRAMP.

Histologic Evaluation. Histologic findings showed that DSS induced marked epithelial destruction, accumulation of red blood cells, and shortening and partial loss of crypt in the colon. In contrast, mCRAMP treatment at the higher dose increased crypt regeneration and restoration of colonic mucosa, which were comparable with the normal mice (Fig. 2) and were evidenced by a marked reduction in the crypt score (Table 1). These findings further indicated the improvement of the colonic pathology by mCRAMP treatment.

MPO Activity. Moreover, the colonic MPO activity, an indicator of neutrophil accumulation, was profoundly increased in colitis animals (Fig. 4A), and mCRAMP at the dose of 5.0 mg/kg per day significantly decreased this activity in the colitis group, whereas no influence was observed at the lower dose.

Fecal Microflora Populations. Upon examination of fecal microflora before and after colitis induction, there were significant increases in the populations of both aerobes and anaerobes in colitis mice (Fig. 5). Administration of mCRAMP at the dose of 5.0 mg/kg per day reversed these increases, and the amounts of both aerobes and anaerobes were comparable before and after colitis induction. In the normal mice without colitis, mCRAMP tended to decrease the populations of aerobes and anaerobes, although no significant change was found (Fig. 5).

Apoptosis. As shown by the TUNEL staining, fewer than five apoptotic cells per field were found in the colon from all normal mice with or without mCRAMP treatment. However, this number increased to 20 cells per field in the colon from the colitis mice without mCRAMP treatment. The higher dose of mCRAMP administration significantly reduced the number of apoptotic cells in these animals (Fig. 7).

Mucus Thickness. The thickness of the mucous-secreting layer in the colon declined significantly during inflammation in colitis mice. Intrarectal administration of mCRAMP during colitis induction significantly increased the thickness of the mucous secreting layer with an effective dose at 5.0 mg/kg per day. In contrast, no effect was found in the normal mice with mCRAMP treatment (Fig. 8).

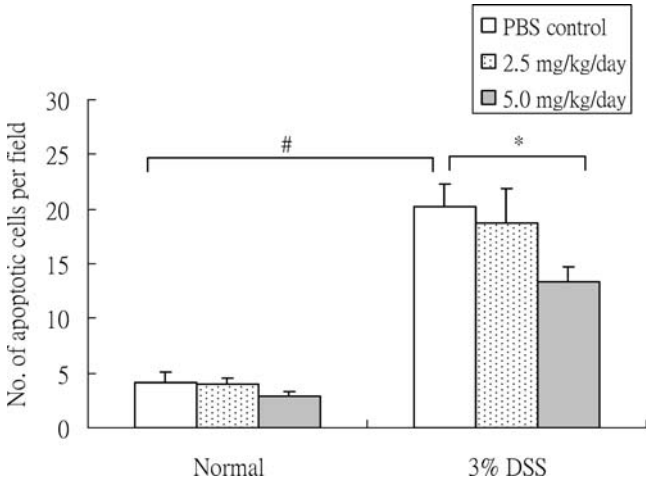

Figure 7. Effects of intrarectal administration of mCRAMP on apoptosis in colonic tissues of mice with parallel DSS treatment. TUNEL staining was performed to visualize the apoptotic cells, and all sections were counted blindly. Results were calculated as the total number of positive cells per field (original magnification ×200). Values are mean ± SE ($n = 12$ in each group). #$P < 0.01$ compared with the normal control group. *$P < 0.05$ compared with the PBS control with DSS administration.

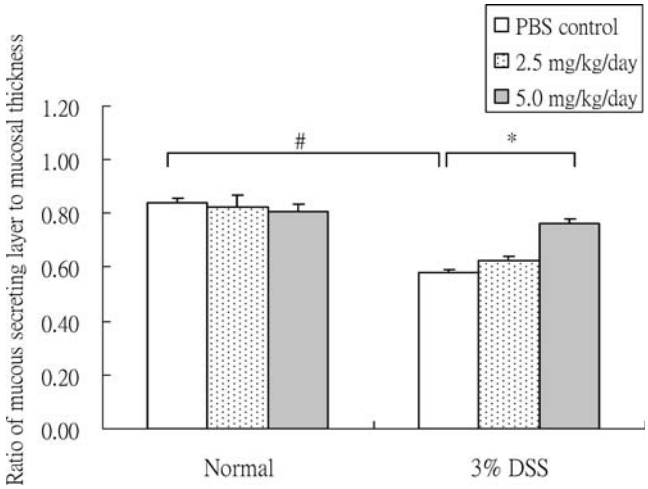

Figure 8. Effects of intrarectal administration of mCRAMP on the mucus-secreting layer in normal or colitis mice with parallel DSS treatment. The results were calculated by determination of the length of the mucus-secreting layer over the total mucosal thickness. Values are mean ± SE ($n = 12$ per group). #$P < 0.01$ compared with the normal control group. *$P < 0.05$ compared with the PBS group with DSS administration.

Mucin Gene Expression. The colonic gene expression of *MUC1*, *MUC2*, *MUC3,* and *MUC4* was monitored and compared between groups using real-time PCR. As shown in Figure 9, all mucin genes were downregulated in colitis mice without mCRAMP treatment compared with the normal animals. Administration of mCRAMP tended to dose-dependently increase the expression of all mucin genes in colitis animals, although significant difference was only observed between the nontreated and 5.0 mg/kg per day mCRAMP-treated mice. In particular, *MUC2* was upregulated to the greatest extent—53% compared with the colitis mice without mCRAMP treatment.

Healing Effects of mCRAMP. The healing effects of mCRAMP also were investigated by intrarectal administration of the peptide to mice for 4 days after colitis induction. Higher doses of mCRAMP treatment significantly reduced the fecal populations of aerobes and anaerobes at Day 11 (Fig. 6). Nevertheless, the disease severity was similar among treated and nontreated mice with colitis (Table 2). Both doses (2.5 and 5.0 mg/kg per day) of mCRAMP showed no significant improvement in either colonic length or MPO activity (Figs. 3B and 4B).

Discussion

Endogenous antimicrobial peptides play an important role in innate immunity. Cathelicidin in particular has been widely revealed in recent years as having pronounced influences in inflammation and wound healing. Cathelicidin regulates these processes by chemoattracting leukocytes (14, 24), inducing cytokine release (25), stimulating angiogenesis (26), and promoting cell proliferation (16). Our previous report had demonstrated that cathelicidin enhanced gastric ulcer healing in rats through the TGF-α–dependent pathway (19).

In the present study, the protective and healing effects of cathelicidin in acute colitis were investigated. We first showed that intrarectal administration of mCRAMP had protective effects in a murine colitis model at the dose of 5.0 mg/kg per day, whereas no observable healing effect was noticed. Administration of mCRAMP during DSS feeding effectively protected mice from colitis development. Fewer mice suffered from weight loss, shortening of colon, gross bleeding, and diarrhea. Treatment with mCRAMP also alleviated neutrophil infiltration in colitis tissues, as reflected by a marked decrease in MPO activity. This would partly explain the anti-inflammatory action of the peptide. Histologic analysis further showed that there was a significant morphologic improvement by mCRAMP. Mice treated with mCRAMP showed better crypt regeneration and milder epithelial destruction (Fig. 2). The differences in protection and healing of mucosal damage indicated that the major function of cathelicidin is to prevent colonic inflammation rather than to assist wound healing where the mucosal damage already exists in the colon. Another possible explanation is that the duration of posttreatment (i.e., 3 days) may be too short to promote healing actions in the colon.

Despite the unknown pathogenesis of IBD, the deleterious effects of enteric bacteria have received much attention in past decades. The involvement of enteric bacteria in IBD is supported by two main observations: (i) lack of intestinal inflammation in animal models kept in germ-free conditions (3), and (ii) increased occurrences of IBDs in patients with high concentration of intestinal bacteria (27). A report by Swidsinski *et al.* demonstrated

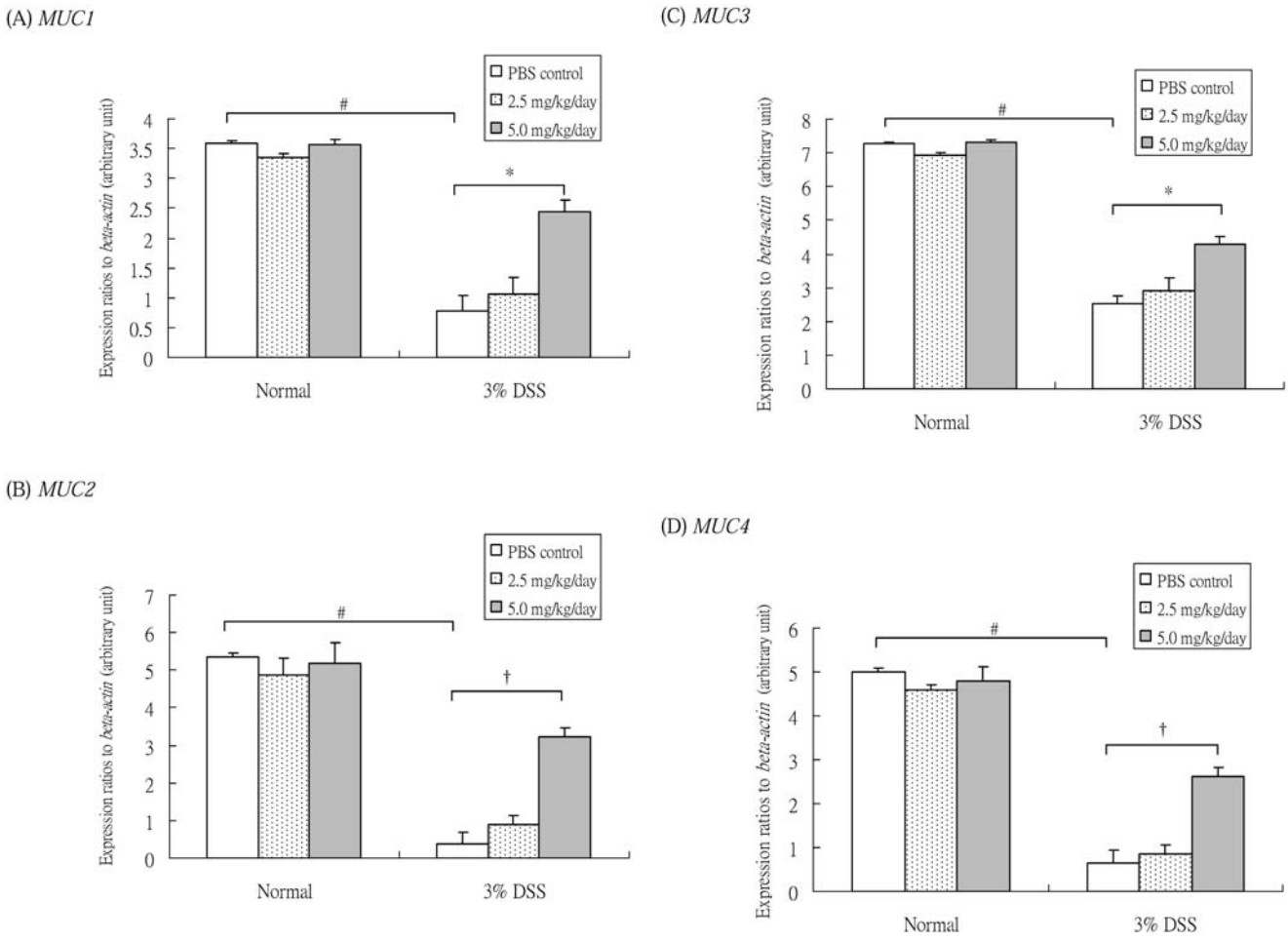

Figure 9. Effects of intrarectal administration of mCRAMP on the expression of four mucin genes in the colonic tissues of mice with parallel DSS treatment. The gene expression of each target gene was determined by real-time PCR, which was standardized against the expression of β-*actin*. Values are mean ± SE ($n = 12$ in each group). #$P < 0.01$ compared with the normal control group. *$P < 0.05$ and †$P < 0.01$ compared with the PBS group with DSS administration.

that the quantity of intestinal bacteria in IBD patients was higher than that in the healthy subjects and increased progressively with the severity of the disease (27). The fecal microflora populations, both anaerobic and aerobic, in mice increased significantly after colitis induction, and mCRAMP administration abrogated these increases. Cathelicidin probably ceased the development of colitis by inhibiting microbial growth in the colon. Less bacterial invasion in the damaged mucosa might improve abnormal immunoresponses induced by DSS in the colon. In contrast, normalization of fecal microflora populations by mCRAMP did not show any healing effects in the colitis animals. This showed

Table 2. Effects of mCRAMP Treatment Given after DSS Administration on Clinical Symptoms in Ulcerative Colitis

	Normal control[a]	3% DSS[a]		
mCRAMP (mg/kg per day)	0	0	2.5	5
Gross bleeding (% animals)	0	50*	67	67
Loose stools (% animals)	0	100*	100	100
Diarrhea (% animals)	0	50*	83	100
Weight loss (% animals)[b]	0	67*	50	83
Disease activity index	0.0 ± 0.00	2.2 ± 0.27*	2.2 ± 0.31	2.2 ± 0.27
Crypt scores	0.0 ± 0.00	1.7 ± 0.25*	1.5 ± 0.29	1.5 ± 0.29

[a] ($n = 8$ per group).
[b] Weight loss defined as a 5% drop in body weight.
* $P < 0.01$ compared with the normal control.

that modification of the number of fecal microflora might not be important in the healing process of mucosal damage in the colon. Instead, it might be vital in the initiation of colitis. Indeed, Cummings *et al*. demonstrated that antibiotic treatment was only effective if given before the onset of colitis (28). This observation is consistent with the present study and suggests that cathelicidin could act like antibiotics to prevent UC.

Although researchers so far have failed to confirm a direct pathogenic role for a specific infectious microbe in UC, an increase was noted in members of Gram-negative anaerobes in experimental colitis induced by DSS (29, 30). Okayasu *et al*. have further shown that *Bacteroides distasonis* and *Clostridium* spp. increased significantly in mice with DSS-induced colitis (31). In this study, we have demonstrated that the fecal amount of anaerobes and aerobes increased substantially after colitis induction, and mCRAMP could reverse these changes. The changes of specific microbes are unknown. Further experiments should be performed in the future to investigate the detailed role of mCRAMP on colitis.

Another finding in this study is the antiapoptotic effect of cathelicidin in the colon. As shown by the TUNEL staining, colitis markedly increased the number of apoptotic cells in the colonic mucosa. Administration of mCRAMP alleviated this pathogenic process in colitis, with no effect in the normal mucosa. It has been shown that apoptosis is one of the ulcerogenic processes in the gastric mucosa (32). Increasing evidence shows that dysregulation of this process in the colonic mucosa is also implicated in the pathogenesis of IBD (33–35). The current study showed that one of the mechanisms for mCRAMP to prevent DSS-induced colitis may be through the inhibition of apoptosis in the colonic mucosa. This would further explain why cathelicidin was more effective in preventing colitis than in promoting colonic healing.

The current study also shows the effect of cathelicidin on mucus synthesis in the colonic mucosa. Mucus is a water-insoluble gel that acts as a physical barrier to defend against injurious agents disturbing the epithelial integrity (10). Indeed, the thickness of the colonic mucus-secreting layer substantially declined by about 28% after colitis induction. Administration of mCRAMP preserved the mucus-secreting layer and significantly reversed the reduction of mucus layer. It is likely that cathelicidin could stimulate the mucus synthesis from the mucus-secreting layer in the colonic mucosa. The colonic epithelium is composed of trefoil peptides, like TFF3, and mucins, including MUC1, MUC2, MUC3, and MUC4, in which MUC2 is the predominant secretary mucin in the colon (36). To further understand the mechanism of mCRAMP in inducing mucus synthesis, the mRNA levels of these genes were measured in colonic tissues using real-time PCR. Results demonstrated that all of the mucin genes were markedly downregulated during colitis. A dose of 5.0 mg/kg per day of mCRAMP significantly upregulated the expression of these genes at the mRNA level, whereas no difference was detected for *TFF3* (data not shown). More importantly, *MUC2* was upregulated to the highest extent, a 53% increase compared with the colitis-induced mice without mCRAMP treatment. A recent *MUC2* knockout mouse model has confirmed that *MUC2* deficiency led to abnormal morphology, ulceration of epithelial cells, and a mild increase of inflammatory cells in the colon (11). The knockout animals developed more serious colitis symptoms by 2.5% DSS feeding for 2 days. *MUC2* seems to be critical for colonic protection. It has been reported that mucus layer in the colon of patients with UC was thinner than in healthy subjects (37), and the reduction of mucus layer in UC patients was linked to the decrease in *MUC2* expression (38). Taken together, cathelicidin may be useful in treating UC patients by boosting mucin gene expression and increasing mucus synthesis in the colonic mucosa, which was perturbed in UC patients.

In summary, the present study shows that intrarectal administration of a synthetic peptide of cathelicidin could mitigate against DSS-induced colitis in the murine model. Cathelicidin has multiple mechanisms of action to prevent colitis development. It has a direct antimicrobial action, antiapoptotic properties, and enhances the mucus barrier. The study by Schauber *et al*. has further proved the importance of cathelicidin in UC in humans (18). In patients who had IBDs, especially UC, the repeated cycle of injury and repair of intestinal mucosa has been reported to increase the risk of colon cancer (8). The blockage of this cycle by prophylactic treatment of UC could overcome the risk of cancer development. Taken together, cathelicidin may be a promising prophylactic therapy for IBDs and colon cancer in the future.

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