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## Regulatory Role of Casein Glycomacropeptide (CGMP) in the Faecal Flora of Mice with Ulcerative Colitis

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### Research Article

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#### ABSTRACT

**Objective:** To explore the regulatory role of Casein glycomacropeptide (CGMP) in the fecal flora of mice with Ulcerative colitis (UC).

**Methods:** The BALB/c mice were divided into a normal control group, a model group, CGMP groups with low doses (5 mg/kg-d), middle doses (50 mg/kg-d) and high doses (500 mg/kg-d), and a Sulfasalazine (SASP) group. Except the normal control group, all mice were treated with Oxazolone (OXZ) by perfusion to induce the UC model. In contrast, the mice in the normal control group and the model group were treated with an equal volume of saline for 7 consecutive days, which the changes in the fecal flora of the mouse intestines were evaluated by Fluorescence in situ hybridization (FISH) and DNA staining.

**Conclusion:** CGMP can rectify the structural imbalances in the colon fecal flora in mice with UC and can improve the proportion of dominant flora in the intestine. CGMP at a dose of 50 mg/kg-d can significantly reduce the number of *Enterobacteriaceae* and *Enterococci* and promote the proliferation of *Bifidobacteria*, *Lactobacilli*, *Bacteroides* and *Clostridium coccoides* in the intestine.

### INTRODUCTION

The intestinal flora is composed mainly of the bacteria in the feces and in the intestinal mucosa. In normal human feces, approximately 80% of the bacteria belong to 6 dominant genera or species: *Bifidobacterium*, *Lactobacillus*, *Bacteroides*, *Clostridium leptum*, *Clostridium coccoides* and *Enterobacter* [1]. The intestinal microbial flora is a dynamic metabolic population that can be beneficial with respect to host metabolites such as short-chain fatty acids and vitamins. Under normal circumstances, this complex micro-ecosystem can prevent pathogens from invading by competing with pathogens for nutrients or for epithelial cell target sites. Inflammatory bowel disease (IBD), including Ulcerative colitis (UC) and Crohn's disease (CD), is a type of metabolic disease whose incidence is highly correlated with intestinal microbial flora, genetics and immune factors [2]. Currently, many experimental and clinical studies have shown that IBD is an inflammatory response in genetically susceptible hosts that is triggered by imbalanced microorganisms and antigens in the intestinal tract [3]. Many microbial antigens, such as enteroaggregative *Escherichia coli*, are thought to be activation factors for IBD [4]. Previous studies have confirmed that these bacteria and their metabolic products can induce imbalances in the immune function of the intestinal mucosa, resulting in the loss of tolerance to antigens in the intestinal mucosal immune system and triggering inflammation [5]. The imbalanced intestinal flora of IBD patients can reduce the levels of probiotics and their metabolites and increase the levels of harmful bacteria and toxic metabolites, thus leading to dysfunctions in the intestinal barrier and bacterial translocation. Meanwhile, microbial antigens in contact with human tissues activate the Toll-like receptor (TLR) and NF- $\kappa$ B signaling pathways, thereby promoting the secretion of inflammatory cytokines and chemokines and finally triggering inflammatory responses [6].

Casein glycomacropeptide (CGMP) is a biologically active peptide derived from  $\kappa$ -casein ( $\kappa$ -CN) in milk. During the practical

preparation of cheese, rennet can hydrolyze the peptide bond between a phenylalanine and methionine of  $\kappa$ -CN to generate insoluble sub- $\kappa$ -CN (amino acids 1-105 of the peptide chain) and the soluble Glycomacropeptide (GMP), which contains 64 amino acids (amino acids 106-169 of the peptide chain)<sup>[4]</sup>. GMP can be detected in separated whey as a byproduct of cheese preparation. In addition, because this polypeptide is derived from casein and contains more sugar chains, it is termed CGMP<sup>[7]</sup>. Sweet whey contains approximately 1.2-1.5 g/L CGMP, which is equivalent to 15-20% of the total whey protein<sup>[8]</sup>. CGMP is rich in branched-chain amino acids and is low in methionine, which makes it ideal for the diets of patients suffering from hepatic diseases<sup>[9]</sup>. In addition, it also can be used as a nutritious protein source for patients suffering from phenylketonuria, as it lacks phenylalanine<sup>[10]</sup>. CGMP also has multiple biological activities, such as antimicrobial activity<sup>[11]</sup>, probiotic proliferation enhancement<sup>[12]</sup>, and immune regulatory activity<sup>[13]</sup>.

To explore the characteristics of microbial communities in complex environments, scientists have established and developed a large number of methods, including cell culture analysis and independent molecular analysis, to investigate micro-ecological environments. However, uncultured microbial molecular-level identification technologies remain mainstream techniques; they include Fluorescence in situ hybridization (FISH), DGGE/TGGE, fluorescence quantitative PCR, 16S rRNA gene Restriction fragment length polymorphism (RFLP) analysis, **Enterobacterial repetitive intergenic consensus** (ERIC)-PCR, gene chips (DNA microarrays), 16S rRNA gene clone library analysis, 454 pyrophosphoric acid sequencing, and the Personal genome machine (PGM) sequencing system. In the present study, FISH combined with DNA staining was used to explore the regulatory role of CGMP in the intestinal fecal microflora of mice with UC. This study will provide a theoretical basis for using CGMP as a nutritional therapy for UC and a feasible strategy for the improvement and regulation of human intestinal diseases using foodborne functional nutrients.

## MATERIALS AND METHODS

### Experimental Animals and Materials

BALB/c mice (SPF grade, male, body weight  $25 \pm 2$  g) and normal feed for mice were purchased from the Experimental Animal Center for the Academy of Military Medical Sciences of the Chinese People's Liberation Army (PLA). CGMP was purchased from the Tatua Co-Operative Dairy Company (Morrinsville, New Zealand). CGMP had a purity of 71% and a sialic acid content of 5.6%. Oxazolone (OXZ) was purchased from Sigma (Sigma, USA). Sulfasalazine (SASP) enteric-coated tablets were purchased from Shanghai Sunway Pharmaceutical Co., Ltd. The experimental probes were ordered from Shanghai Yingjun Biological Technology Co., Ltd. (Invitrogen).

### Experimental Methods

#### *Experimental animal grouping*

After adaptive feeding for 1 week, a total of 90 BALB/c mice were randomly divided into 6 groups: a normal control group, a model group, and three CGMP groups at doses of 5, 50 or 500 mg/kgd, and a SASP group at dose of 40 mg/kgd. Each group included 15 animals.

#### *Preparation of mouse UC model*

Hair was removed from the abdomens of the mice by shaving a 2 cm  $\times$  2 cm patch of skin. An allergic reaction was induced by applying 3% OXZ for 2 days; the mice were prevented from eating for 3 h but were allowed free access to water beginning the night after allergy induction. On the 6<sup>th</sup> day, a silica-gel tube was inserted into the colon 4 cm from the anus. The mice in the normal control group were subjected to perfusion of 0.15 mL of 50% ethanol. The mice in the other groups were subjected to perfusion with the same volume of 1% OXZ (OXZ dissolved in 50% ethanol).

#### *Gavage*

After successful model establishment, the mice in the normal control group and in the model group were treated with saline. The mice in the CGMP groups were treated with 5, 50 or 500 mg/kgd of CGMP, and the mice from SASP group were treated with 40 mg/kgd of SASP by gavage. In each group, the treatment lasted for 7 consecutive days.

#### *The role of CGMP in the intestinal flora of mice with UC analyzed by FISH combined with DNA staining*

Approximately 0.3 g of stool was harvested from the mice in each group on day 0 and on the 3<sup>rd</sup>, 5<sup>th</sup>, 7<sup>th</sup>, 11<sup>th</sup> and 21<sup>st</sup> days and separately placed in 5-mL RNase-free centrifuge tubes. Next, 2.7 ml of filter-sterilized (0.22 $\mu$ m membrane) PBS and a small amount of 4mm glass beads were added to the tubes. After full whirlpool shaking, the samples were centrifuged at 700  $\times$  g at 4 $^{\circ}$ C for 1 min. A total of 1 ml of supernatant was then diluted 10-fold using 9 ml of sterile saline. After shaking and mixing, the bacterial suspension was mixed with a 1/3 volume of 4% paraformaldehyde (PFA), and the bacteria were fixed and the bacteria were fixed at 4 $^{\circ}$ C for 16 h. A total of 10  $\mu$ l of lysozyme was added to the bacteria, and the bacterial suspensions were incubated at 37 $^{\circ}$ C for 45 min. The bacterial suspension was appropriately diluted for effective microscopy, and 10  $\mu$ l of the diluted bacterial solution was evenly applied to slides (poly-L-lysine treated, with a plaque diameter of approximately 1 cm). The slides were then subjected to dehydration in 50%, 75% and 95% ethanol for 3 min and were air dried. Preheated hybridization buffer containing 20 ng/ $\mu$ l of the corresponding probe (probes are shown in **Table 1**) was added to the slides in the dark. The samples were covered

with RNase-free coverslips for hybridization in a wet box at 46 °C for 16 h. The slides were then soaked in preheated wash buffer at 48 °C for 30 min to remove non-hybridized probes. After washing the slides with distilled water and drying, DAPI (4',6-diamidino-2-phenylindole, 4',6-linked amidino-2-phenylindole dihydrochloride) was added to the slides in the dark at room temperature to stain DNA. Finally, the slides were washed with distilled water and air dried. Approximately 10-20 fields were randomly selected for bacterial count analysis using the DS-5MC fluorescence microscopy system and the NIS-Elements BR imaging analysis system.

**Table 1.** Sequences of 16S rRNA probes.

Probe	OPD code	Probe sequence (5' - 3')	Target organism
Enter1432	S-G-Enter-1432-a-A-15	GTTTTGCAACCCACT	Enterobacteriaceae
Bif164	S-G-Bif-0164-b-A-18	CATCCGGYATTACCACCC	Bifidobacterium
Lacb722	S-G-Lacto-722-a-A-25	YCACCCTACACATGRAGTTCCACT	Lactobacillus
ENC221	not applicable	CACCGCGGTCCATCCATCA	Enterococcus spp
Bac303	S-*-Bacto-0303-a-A-17	CCAATGTGGGGGACCTT	Bacteroides-Prevotella
Erec482	S-*-Erec-0482-a-A-19	GCTTCTTAGTCARGTACCG	Clostridium coccoides Eubacterium rectale

Note: R=G/A, Y=T/C, M=A/C, K=G/T, S=G/C, W=A/T, H=A/C/T, B=G/T/C, V=G/C/A, D=G/A/T, N=G/A/T/C.

### Optimization of DNA staining conditions

Using the plaque size on the slides as described above (approximately 1 cm in diameter), 3 factors, namely DAPI amount, dye concentration and staining duration, were investigated to explore the optimal DNA staining conditions by using orthogonal tests with the 3 factors at 3 different levels. The factors and levels used in the orthogonal tests for optimizing DNA staining are shown in **Table 2**.

**Table 2.** Factors and levels of orthogonal tests for DNA staining (L9 (34)).

Factors	Levels		
	1	2	3
DAPI amount, A (μl)	10	20	30
Dye concentration, B (μg/ml)	5	50	500
Staining time, C (min)	15	30	45

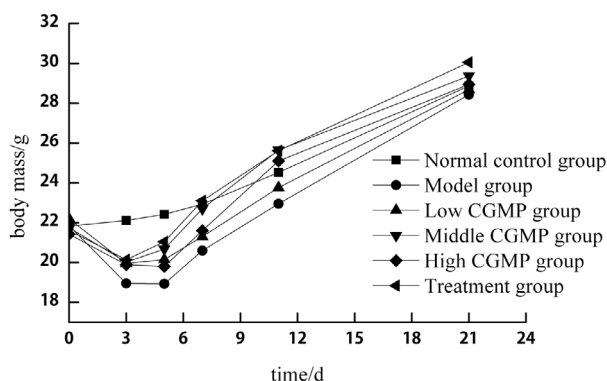
### Statistical analysis

All experimental data were subjected to processing using SPSS11.5 statistical software. All data are expressed as the mean ± standard deviation (Mean ± SD).

## RESULTS AND ANALYSIS

### General Status and Body Weight Changes of Mice

As shown in **Figure 1**, during the first 3 days of the experiment, the body weights of the mice in the normal control group increased; however, the mice in the model group exhibited less activity, reduced food consumption, loose hair without luster, some extent of diarrhea, anal adhesion, and a significant reduction in body weight when compared to mice in the normal control group ( $P < 0.01$ ). Although the body weights of the mice in the 3 CGMP groups and in the SASP treatment group showed a smaller decline than that of the model group, an obvious difference from the normal control group was observed. In contrast, after 5 days, the mice in the model group, the CGMP groups and the SASP group began to show smooth and lustrous hair, increased food consumption and improved body weights. At the end of the experiment, the general status of the mice in each group was similar.



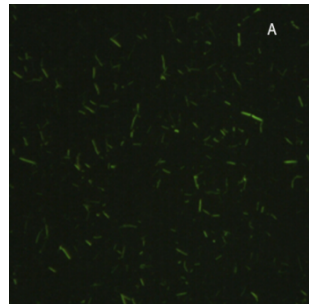
**Figure 1.** Change in body weight in mice from various groups.

### Detection Efficiency of Colon Fecal Flora by FISH Combined with DNA Staining

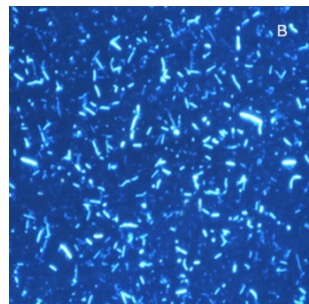
FISH combined with DNA staining was used to analyze the structural changes in the intestinal microbial community in mice

with UC and subjected to CGMP intervention. The DNA staining conditions were optimized using orthogonal tests. The optimal DNA staining conditions required a dye concentration of 500 µg/mL, a DAPI loading volume of 20 µl and a staining duration of 30 min.

A quantitative analysis of lactic acid bacteria in feces was conducted by FISH combined with DNA staining; representative results using the Lacb722 probe are shown in **Figures 2 and 3**.



**Figure 2.** The genus positive hybridization results of intestinal *Lactobacillus* and Lacb722 probe.



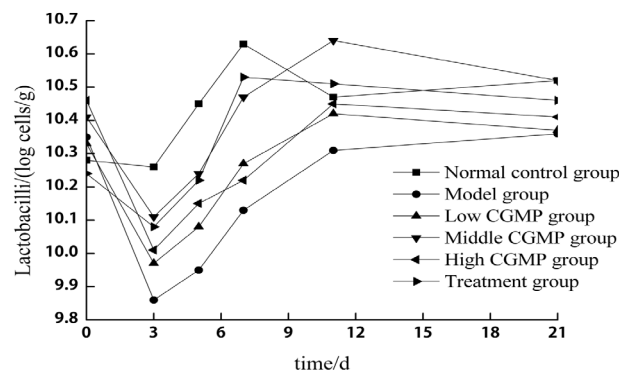
**Figure 3.** Epifluorescence images of intestinal *Lactobacillus* in UC mice using FISH and DNA-staining.

Based on the analysis using optimal DNA staining conditions, this technology is able to clearly reflect structural changes in the intestinal microflora of mice with UC and subjected to CGMP intervention.

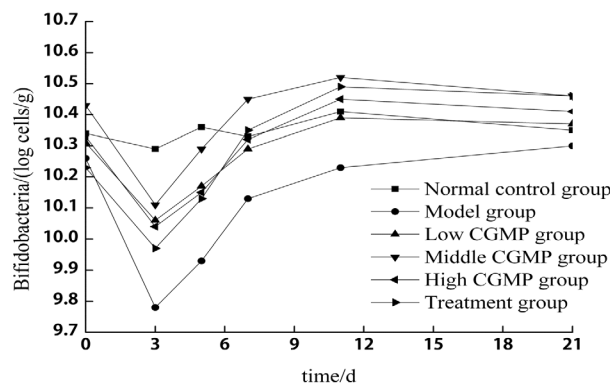
### Effects of CGMP on the Colon Fecal Flora Structure in Mice with UC

#### Effects of CGMP on colon *Lactobacilli* and *Bifidobacteria* in mice with UC

Using optimized conditions for FISH combined with DNA staining, quantitative analysis of intestinal *Lactobacilli* and *Bifidobacteria* in mice with UC was performed; the results are shown in **Figures 4 and 5**.



**Figure 4.** The effects of CGMP on intestinal *Lactobacillus* number of UC mice in each group.

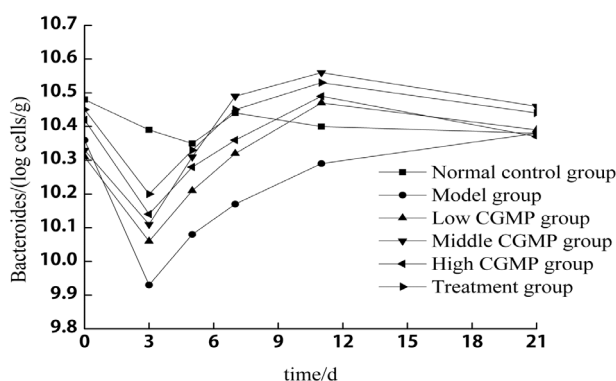


**Figure 5.** The effects of CGMP on intestinal *Bifidobacterium* number of UC mice in each group.

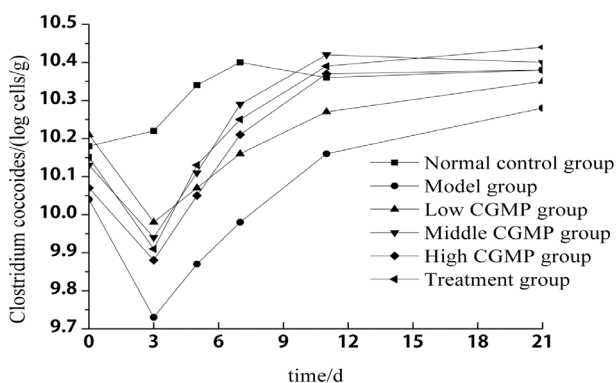
As shown in **Figures 4 and 5**, similar changes in intestinal *Bifidobacteria* and *Lactobacilli* were observed in mice with UC and subjected to CGMP intervention. Generally, CGMP promotes the proliferation of probiotics in the mouse intestine. At the beginning of the study (day 0), the number of intestinal *Lactobacilli* and *Bifidobacteria* in the experimental groups were similar and did not reveal obvious differences. However, on the 3<sup>rd</sup> day of the experiment, the number of *Lactobacilli* and *Bifidobacteria* in the intestines of mice from all of the groups except the control group had significantly decreased, suggesting that UC reduces the dominant microflora. The number of *Lactobacilli* in mice in the model group displayed the most obvious reduction and was significantly different from that of the mice in the middle and high-dose CGMP groups and in the SASP treatment group ( $P < 0.01$ ). The reduction of *Bifidobacteria* in mice in the CGMP groups and the SASP treatment group was also significantly different when compared with mice from the model group ( $P < 0.01$ ). After the 5<sup>th</sup> day of CGMP intervention, there was a slow increase in *Lactobacilli* and *Bifidobacteria* in the CGMP and SASP treatment groups. However, the numbers of *Lactobacilli* after CGMP intervention at both the low and high doses were significantly different compared with the normal control group ( $P < 0.01$ ). The numbers of *Bifidobacteria* in the CGMP and SASP treatment groups on the 7<sup>th</sup> day were clearly higher than that of the model group. The number of *Bifidobacteria* in mice with UC in the middle-dose CGMP group was close to that of the normal control group on the 5<sup>th</sup> day of the experiment. Moreover, the intestinal *Lactobacilli* and *Bifidobacteria* in mice with UC in the three CGMP groups and the SASP treatment group clearly increased and were significantly different from the normal control group and the model group ( $P > 0.05$ ). Therefore, oral administration of CGMP in mice with UC can promote the proliferation of intestinal *Lactobacilli* and *Bifidobacteria*. In addition, CGMP treatment at an appropriate concentration plays a regulatory role in the intestinal flora of mice with UC that is similar to SASP treatment ( $P > 0.05$ ).

#### Effects of CGMP on the populations of *Bacteroides* and *Clostridium coccoides* in the intestines of mice with UC

Using the optimal conditions for FISH combined with DNA staining, a quantitative analysis of intestinal *Bacteroides* and *Clostridium coccoides* in mice with UC was performed; the results are shown in **Figures 6 and 7**.



**Figure 6.** The effects of CGMP on intestinal *Bacteroides* number of UC mice in each group.

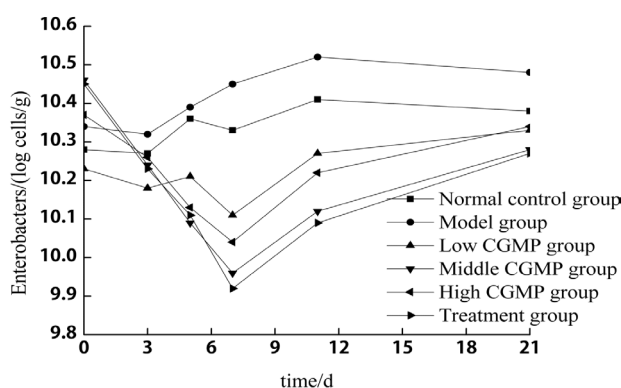


**Figure 7.** The effects of CGMP on intestinal *Clostridium coccoides* number of UC mice in each group.

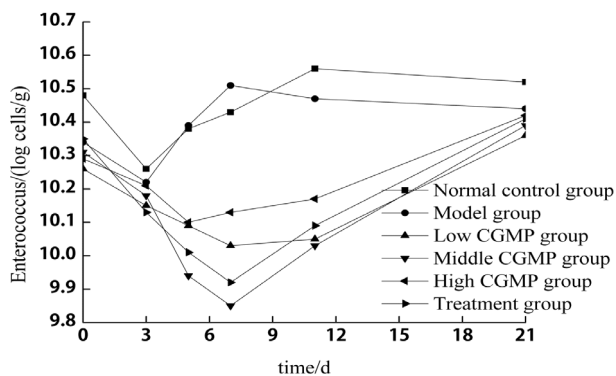
**Figures 6 and 7** show the changes in both microflora. At the beginning of the experiment, the number of *Bacteroides* and *Clostridium coccoides* was similar across all groups. After establishing the UC model for 3 days, the number of *Bacteroides* in the model group significantly decreased, suggesting that UC results in an imbalance of the intestinal flora in mice. On the 5<sup>th</sup> day of the experiment, the number of intestinal *Bacteroides* and *Clostridium coccoides* increased in each group of mice. The number of *Bacteroides* in the mice from the middle- and high-dose CGMP groups and in the SASP treatment group significantly increased compared with the mice in the model group ( $P < 0.01$ ). Similarly, the number of *Clostridium coccoides* in the mice in the 3 CGMP groups and in the SASP treatment group displayed an upward trend and revealed significant differences compared with the mice in the model group ( $P < 0.05$ ). These results suggest that CGMP improves intestinal *Bacteroides* and *Clostridium coccoides* levels in mice with UC. On the 7<sup>th</sup> day of the experiment, the number of intestinal *Bacteroides* in the mice in the middle-dose CGMP group was higher than that in the SASP treatment group and was not obviously different from the normal control group. Moreover, the numbers of intestinal *Bacteroides* and *Clostridium coccoides* reached similar levels, without obvious differences, even after terminating gavage for 2 weeks.

**Effects of CGMP on the populations of Enterobacteriaceae and Enterococci in the intestines of mice with UC**

Using the optimal conditions for FISH combined with DNA staining, a quantitative analysis of intestinal *Enterobacteriaceae* and *Enterococci* in mice with UC was performed; the results are shown in **Figures 8 and 9**. As shown in **Figures 8 and 9**, the number of *Enterobacter* and *Enterococcus* was initially similar across all groups. After CGMP and SASP were orally administered to mice for 3 consecutive days, mice in the normal control group and the low-dose CGMP group, and especially in the model group, displayed an obvious increase in *Enterobacteriaceae*. However, the numbers of *Enterobacteriaceae* in mice in the middle and high-dose CGMP groups and in the SASP treatment group displayed a downward trend on the 3<sup>rd</sup> day of the experiment and a highly significant decrease compared with the model group on the 5<sup>th</sup> day of the experiment ( $P < 0.01$ ). After 1 week of treatment, the numbers of intestinal *Enterobacter* in the mice in the 3 CGMP groups and in the SASP treatment group reached their lowest levels, which were significantly different from the normal control group ( $P < 0.01$ ). In addition, the numbers of intestinal *Enterobacteriaceae* in the 3 CGMP groups were higher than that of the SASP treatment group, indicating that an appropriate dose of CGMP can inhibit the proliferation of intestinal *Enterobacteriaceae* [15]. Similarly, as shown in Figure 8, the numbers of intestinal *Enterococci* in mice in the 3 CGMP groups and in the SASP treatment group also displayed a downward trend and were significantly different from the model group after oral administration for 3 days ( $P < 0.05$ ). On the last day of gavage, the numbers of *Enterococci* in the treatment groups were significantly lower than those of the normal control group or the model group ( $P < 0.01$ ). Moreover, the middle dose of CGMP displayed changes in the number of fecal *Enterococci* similar to those seen in the SASP treatment group on the 5<sup>th</sup> day of the experiment; although the number of *Enterococci* in mice in the middle-dose CGMP group was lower than that in mice in the SASP treatment group. Therefore, CGMP can reduce the number of *Enterococci* in mice with UC.



**Figure 8.** The effects of CGMP on intestinal Enterobacter number of UC mice in each group.



**Figure 9.** The effects of CGMP on intestinal Enterococcus number of UC mice in each group.

**DISCUSSION**

The pathogenesis of IBD remains unclear. Current scientific research has found that the intestinal microflora is closely correlated with the generation and development of IBD in addition to genetic and environmental factors. Previous reports have revealed that the occurrence of IBD is an inflammatory immune response that is due to imbalances in the intestinal microflora and is triggered by genetically susceptible hosts or environmental factors [2]. Genetically deficient mice, such as IL-/- mice, will not develop colitis under sterile conditions, suggesting that the intestinal microflora plays an important role in the development of IBD [14]. Clinical studies have also found that the inflammation sites of IBD are mainly located in the intestinal sections with the highest concentrations of intestinal microflora. In addition, the intestinal mucosa of IBD patients has high permeability, which results in enhanced absorption of microbial antigens and their metabolites [15]. Other studies have demonstrated that IBD, as a chronic metabolic disease, is due to imbalances in the intestinal flora, indicating that the intestinal flora is the trigger of IBD [16]. Imbalances in the intestinal flora can cause declines in the proportion of commensal intestinal flora, thus increasing the proportion of pathogenic bacteria attached to the intestinal mucosa [17]. Numerous studies, including work from our laboratory [18] have shown that the intestinal flora induces IBD in two major ways, namely by promoting the proliferation of inflammatory bacteria and by inhibiting the generation of probiotics and their metabolites.

Currently, investigations around the world have demonstrated the importance of the intestinal flora in maintaining intestinal health. Therefore, it is important to develop intestinal microflora detection technology that is simple to operate and allows for high-throughput screening, high accuracy and systematic analysis. Such technology will aid scientific research and clinical applications and will play a critical role in improving the effectiveness of drugs such as antibiotics, functional health ingredients, and nutrients that generate and develop intestinal flora. In addition, such technology has important scientific value for understanding the temporal patterns of the microfloral population in the human intestinal tract. Favier et al. <sup>[19]</sup> have used the PCR-DGGE technique to monitor the succession of intestinal microflora in healthy infants over 10 consecutive months. Their results showed that the PCR-DGGE technique can complete a dynamic description of infant intestinal microflora. The DGGE band type is simple on the first day after birth, but the diversity of intestinal flora increases over time, and this technique also detected undiscovered or uncultured flora. Gueimonde et al. <sup>[20]</sup> have employed Q-PCR and FISH techniques to determine the number of *Bifidobacteria* in feces from healthy persons of various ages. Both techniques provide an accurate quantitative analysis of *Bifidobacteria* at high concentrations and show excellent correlation. In addition, Q-PCR was much more sensitive than FISH technology. Niess et al., <sup>[21]</sup> have used flow cytometry to analyze the effects of symbiotic bacteria on the expression of proinflammatory CD4<sup>+</sup> T-cells in the mouse colonic lamina propria under normal and inflammatory conditions. Under inflammatory conditions, CD4<sup>+</sup> T-cells display elevated spontaneous secretion of the pro-inflammatory cytokine IL-17. The elevated IL-17 level in the serum is thought to be the result of aggravated inflammation. Moreover, the intestinal flora is a requirement for CD4<sup>+</sup> T-cell-related cytokine accumulation. Cao <sup>[22]</sup> used ERIC-PCR techniques to explore the effects of CGMP on the intestinal microbial community structure and its dynamic changes in mice. The results showed that CGMP can significantly increase the diversity of the mouse intestinal flora. The dominant flora in the mouse intestine benefits the stability of the microbial community structure. Vaahtovuori <sup>[19]</sup> applied a FCM-FISH technique for the quantitative analysis of fecal bacteria and a DNA staining technique for detecting the number of intestinal flora that is more sensitive than Eub338 probe hybridization technology. DAPI-based DNA staining can penetrate a disrupted membrane to stain nuclei and generate more than 20-fold stronger blue fluorescence than its native fluorescence, thereby increasing the efficiency and sensitivity by which bacteria are detected. In the present study, FISH was combined with DNA staining to explore the regulatory role of CGMP in the intestinal microbial flora of mice with UC. Based on the results of this study, FISH combined with DNA staining can clearly evaluate the ratio and distribution of FISH-hybridized cells among the intestinal flora. Moreover, this technology also allows for more intuitive observation, more convenient counting, and improved detection accuracy of the intestinal fecal flora. In particular, these results are consistent with detection by full-sequencing technologies (ion torrent PGM) for intestinal fecal microflora, which we used in our previous reports. Additionally, the methods used in this study represent a feasible way to develop a new detection strategy for scientifically and accurately exploring the relationship between the intestinal microbial community and human health.

Changes in the intestinal microflora can increase the permeability of the intestinal mucosa, resulting in bacterial translocation and thereby promoting an intestinal inflammatory response by activating macrophages and dendritic cells and generating proinflammatory cytokines <sup>[23]</sup>. In this study, the UC mouse model displays imbalances in the intestinal microflora, such as clearly reduced proportions of probiotics and significantly increased numbers of harmful bacteria such as *Enterobacteriaceae* and *Enterococci* ( $P < 0.01$ ). Our results have confirmed that CGMP plays a role in regulating the intestinal microflora in OXZ-induced mice with UC by promoting the proliferation of *Lactobacilli* and *Bifidobacteria*, suppressing the growth of *Enterobacteriaceae* and *Enterococci*, and enhancing the proliferation of *Bacteroides* and *Clostridium coccoides*. Swidsinski et al. <sup>[24]</sup> previously used FISH to analyze the intestinal microflora of IBD patients and revealed that the number of *Bifidobacteria* in the intestines of IBD patients was significantly lower than in healthy persons. The number of *Enterobacter* in the intestines of CD patients is increased, while the number of *Bacteroides* in UC patients is lower than in CD patients, suggesting that UC and CD have different dominant microflora.

Sokol et al. <sup>[25]</sup> have used FISH and FCM technology to analyze the composition of the intestinal flora in IBD patients. The proportion of *Clostridium coccoides* in IBD patients is decreased compared to that of healthy individuals ( $P < 0.01$ ). We have reached conclusions that are in accord with previous reports. However, *Enterococci* are opportunistic pathogens, and their role in the pathogenesis of IBD is still controversial. Some studies have shown that *Enterococci* probiotics can improve the immune response of poultry. Similarly, Suvorov and colleagues have also reported the application of *Enterococci* as "auto-probiotic" strains for the successful treatment of IBD patients <sup>[26]</sup>. In our laboratory, Cao <sup>[27]</sup> has shown that CGMP can promote the proliferation of probiotics in normal mice and inhibit the growth of pathogenic bacteria. In the present study, CGMP regulated the intestinal microflora in mice with UC; the middle dose (50 mg/kg) of CGMP displayed the most obvious regulatory role and did not significantly differ from SASP treatment.

## CONCLUSION

In this study, based on the results of FISH detection combined with DNA staining, CGMP plays a regulatory role, correcting imbalances in the intestinal flora by promoting the proliferation of intestinal *Lactobacillus*, *Bifidobacterium*, *Bacteroides* and *Clostridium* probiotics, inhibiting the growth of harmful bacteria such as *Enterococci* and *Enterobacter*, and protecting the intestinal barrier function. Therefore, the milk-derived bioactive peptide CGMP is a functional health product that may be useful for rectifying the imbalance of intestinal flora in metabolic diseases.



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