OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2018.

Research Article
Orally Administered Chitooligosaccharides Modulate Colon Microbiota in Normal and Colitis Mice

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Abstract

Background and Objective: The alterations in the gut microbiota composition are gaining increasing attention in view of their influence on the development of ulcerative colitis. The anti colitis effect of orally administered chitooligosaccharides (COS) had been reported in the animal models of ulcerative colitis but the mechanism is still uncertain. Interestingly, COS have long been proposed as potential natural prebiotics based on *in vitro* experiments. The aim of this study is to confirm the prebiotic property of COS *in vivo* and further clarify the mechanisms of their anti colitis effect. **Materials and Methods:** The COS at the dose of 500 mg kg^{-1} were orally given normal mice and colitis mice treated by 3.5% dextran sulfate sodium (DSS). The colon microbial composition in mice was evaluated by qualitative analysis of 16S ribosomal DNA in colonic content samples using real-time PCR. **Results:** The COS could function as prebiotics by increasing the levels of Bacteroidetes and Actinobacteria phyla, the relative ratio of Bacteroidetes to Firmicutes, as well as common probiotics such as *Lactobacillus* and *Bifidobacterium* and inhibiting the growth of Firmicutes and Proteobacteria phyla, as well as potential pathogens such as *Enterococcus*, in both normal and colitis mice. In addition, oral intake of COS were found to enhance the colonic concentrations of short-chain fatty acids (SCFAs), the dominating fermentation end-products of bacteria in the large bowel having abilities to support the transport processes, energy metabolism, cellular growth and differentiation of colonocytes. **Conclusion:** The data suggested that COS administration might had beneficial effects on the health of the intestinal tract and more importantly, tended to protect mice from dysbiosis of native gut microbiome and against the suppression of SCFA production, which might be a potential mechanism for their anti colitis effect.

Key words: Chitooligosaccharides, ulcerative colitis, gut microbiota, short-chain fatty acids

Received: Accepted: Published:

Citation: Ting Long, Zhi-Jun Yu, Jun Wang, Jia Liu and Bing-Shu He, 2018. Orally administered chitooligosaccharides modulate colon microbiota in normal and colitis mice. Int. J. Pharmacol., CC: CC-CC.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Ulcerative colitis (UC), one of major forms of inflammatory bowel disease, is a non-specific chronic relapsing inflammatory disorder of colonic mucosa, which has been increasingly prevalent around the world. The typical symptoms of UC include bloody diarrhea, urgency and tenesmus, weight loss, abdominal pain and cramping, which are associated with the significant reductions of patients' quality of life and daily functioning[1,2]. Up to date, the exact pathogenesis of UC remains unknown. But it has been recognized as a multifaceted disorder, in which many exogenous and endogenous factors, such as germline genetics, immune system and colonic environment are all involved[3,4]. Recently, the alterations in the gut microbiota composition are gaining increasing attention in view of their influence on UC development[5,6].

Numerous studies[5,7-9] have corroborated evidence for the disruption of gut microbial homeostasis in patients with UC, which is characterized by a distorted, decreased bacterial diversity. Dysbiosis of the native gut microbiome accompanied with the breakdown of host-microbial mutualism, which may be triggered by host genetics and some environmental factors such as unhealthy diet, is considered as a defining event in the disease progression and severity and the heart of inflammatory process of UC[6,7]. Gut microbiota plays a key role in immune-regulatory and anti-inflammatory functions and a depletion of beneficial bacteria and/or an increase in pathogenic bacteria in gut contributes to the initiation and perpetuation of chronic colon inflammation in UC[10,11].

Under circumstance that benefits of standard UC managements including immunosuppressive and/or anti-inflammatory drugs or surgical intervention were uncertain[2], alternative treatment approaches targeting restoration of the gut microbiota by modifying their composition and overcoming gut dysbiosis have recently been demonstrated potential in laboratory and clinical settings and been believed as a treatment option in UC patients[1,10]. In some studies[12,13], the use of antibiotics was reported to improve clinical outcomes of patients with inflammatory bowel disease including UC. Fecal microbiota transplantation from healthy donors, the aim of which is efficient colonization of the recipient's gut by the donor microbiota, has been used and reported to contribute to disease remission in patients with UC refractory to standard therapies[4,14]. Probiotics, as beneficial microorganisms having the abilities to alter gut microbial diversity through the competitive inhibition of other microbes, increase colonic mucosal barrier function and modulate the gut immunity, could facilitate and stabilize clinical remission in patients with UC[1,6]. As an alternative, the application of prebiotics, the non-digestible carbohydrates favoring the growth or promoting the activity of beneficial bacteria in colon, is an emerging field to combat UC[15,16]. Some prebiotics, such as psyllium, oligofructose-enriched inulin, or germinated barley foodstuff, have been found to provide some benefits in patients with active UC or UC in remission without undesirable side effects[15,16]. These prebiotics could modulate the numbers and composition of gut microbiota, alter the metabolic properties of gut microbiota and improve the survival of probiotics. Through modulating the production of short-chain fatty acids (SCFAs) and then lowering pH values of the colonic environment, prebiotics have the ability to reduce the amount of potentially pathogenic microorganisms[15,16].

Chitooligosaccharides (COS), as the glucosamine oligomers with high absorption rate, good solubility and non-cytotoxicity produced from chitosan in the exoskeleton of crustaceans and the cell wall of marine zoo-plankton, has been attached great attention because of their numerous biological activities[17,18]. In particular, many previous studies[19-21] have demonstrated the anti colitis effect of orally administered COS in the animal models of UC and the mechanisms of which are, without exception, associated with the anti-inflammatory property of COS. The administration of COS after colitis induction has been found to be effective in ameliorating the inflammation of colonic mucosa, reducing the levels of pro-inflammatory cytokines (tumor necrosis factor-α and interleukin-6)[19-21] and inhibiting the activation of inflammatory signalling molecules, such as nuclear factor-kappa B[19-21], cyclooxygenase-2[19-21], inducible nitric oxide synthase[19] and toll-like receptor 4[21]. Interestingly, COS have long been proposed as potential natural prebiotics, since they were found to have a uniqueness that is the ability to reduce the growth rate of pathogenic bacteria and stimulate the growth of some health promoting enteric bacteria[22-24]. Despite the fact that these previous researches on the prebiotic property of COS were all based on *in vitro* experiments, it can be speculated that COS might modulate colon microbiota composition and help to maintain colonic function, which contributes to their anti colitis effect as an important mechanism in addition to their reported anti-inflammatory activity. Therefore, in order to confirm the prebiotic property of COS *in vivo* and further clarify the mechanisms of their anti colitis effect, in the present study, the ability of COS to modulate the colon microbial composition in normal and dextran sulfate sodium (DSS) induced colitis mice was evaluated by qualitative analysis of 16S ribosomal DNA (rDNA) in colonic content samples using real-time PCR.

MATERIALS AND METHODS

Chemical: The COS, prepared by enzymatic hydrolysis from chitosan in crustaceans shell, were obtained from Qingdao BZ Oligo Biotech Co., Ltd., China. The degree of deacetylation is 90% and average molecular weight is 1500 Da. The purity of COS achieved 90% at least, which was determined by HPLC.

Mice and grouping: Female 8 weeks old C57BL/6J mice with the weight of 20±2 g were chased from Laboratory Animal Center of Hubei Province, Wuhan, China. After acclimatization to the laboratory conditions for 1 week, animals were randomly divided into the following 4 groups of 10 mice each: (1) Normal group, (2) COS treated group, orally treated with a single dose of 500 mg kg^{-1} COS suspended in the distilled water once per day for 2 weeks, (3) Colitis group and (4) COS treated colitis group, pretreated with 500 mg kg^{-1} COS for 1 week prior to induction of colitis, then treated with the same dose of COS for another 1 week. Mice in 1 and 3 groups were orally treated with the same volume of distilled water. All animals were fed a commercial diet purchased from the Laboratory Animal Center of Hubei Province. The animal protocols were approved by Laboratory Animal Ethical Committee of Wuhan University of Science and Technology.

Induction of colitis: In order to induce experimental colitis, mice in 3 and 4 groups were given 3.5% (w/v) DSS with a molecular weight of molecule weight 36-50 kDa (MP Biomedicals, Aurora, USA) dissolved in their drinking water for 7 consecutive days. The mice in 1 and 2 groups were fed the normal drinking water without DSS.

Colitis evaluation: Body weight loss, stool consistency/diarrhea and presence of rectal bleeding were daily monitored from the beginning of DSS challenge to the end of the experimental period. Colitis severity was evaluated with the disease activity index (DAI) as described previously[25]. Mice were sacrificed 1 h after the last administration of COS. Colonic damage was evaluated from the length of entire colon (from ileocecal junction to anal verge). Tissue samples of the distal colon were stained with hematoxylin and eosin (HE) for histological examination. The histological activity index for assessing the epithelial damage and the inflammatory cell infiltration was calculated by two independent pathologists as previously described[26].

Bacterial DNA extraction from mice colonic contents: To analyze the gut microbiota, the total colonic contents were collected immediately after the mice were sacrificed. Then total bacterial DNA was extracted from colonic contents using the bacterial DNA isolation kit (Foregene, China) according to the manufacturer's instructions.

Quantitative PCR of gut bacterial DNA: To detect the composition of the bacterial present in colonic contents of the mice, extracted bacterial DNA was submitted to quantitative PCR and amplified using primers (Sangon Biotech Co., Ltd., Shanghai, China). Sequences of the primers were shown in Table 1.

Then 25 μL PCR reactions were set up containing 2 μg of template DNA, 12.5 μL SYBR Green reaction mix (Thermo Fisher Scientific, USA), 0.5 μL of each primer at a concentration of 10 μM and 9.5 μL of nuclease-free water. Quantitative PCR was performed on the Mx3000P real-time PCR system (Stratagene, USA) using the following conditions: One cycle at 95°C for 3 min, then 40 cycles at 95°C for 15 sec and 61.5°C for 30 sec and 70°C for 20 sec, followed by a dissociation stage at 65°C for 31 sec and cycles of 5 sec starting at 65°C, raising 0.5°C per cycle, to obtain melting curves for specificity analysis.

Short-chain fatty acids (SCFA) quantification in colonic content: The concentration of SCFA in the supernatant of the colonic content samples was determined by gas chromatography as previously described[27]. Results were expressed as μmol of SCFA/g of colonic content.

Statistical analysis: Results were presented as mean±standard deviation (SD) and analyzed using SPSS 12.0. Differences between groups were analyzed by one-way analysis of variance (ANOVA). A p-value less than 0.05 was considered statistically significant.

Table 1: Primers used in quantitative PCR of gut bacterial DNA

Target bacterial group/gene	Primer Sequence (5'→3')
Pan-bacteria	F: GCAGGCCTAACACATGCAAGTC
	R: CTGCTGCCTCCCGTAGGAGT
Bacteroidetes	F: CRAACAGGATTAGATACCCT
	R: GGTAAGGTTCCTCGCGTAT
Firmicutes	F: TGAAACTYAAAGGAATTGACG
	R: ACCATGCACCACCTGTC
Actinobacteria	F: TACGGCCGCAAGGCTA
	R: TCRTCCCCACCTTCCTCCG
Gamma proteobacteria	F: TCGTCAGCTCGTGTYGTGA
	R: CGTAAGGGCCATGATG
Bifidobacterium Genus	F: GCGTGCTTAACACATGCAAGTC
	R: CACCCGTTTCCAGGAGCTATT
Lactobacillus group	F: AGCAGTAGGGAATCTTCCA
	R: CACCGCTACACATGGAG
Escherichia coli sub-group	F: CATGCCGCGTGTATGAAGAA
	R: CGGGTAACGTCAATGAGCAAA
Enterococcus spp.	F: CCCTTATTGTTAGTTGCCATCATT
	R: ACTCGTTGTACTTCCCATTGT

RESULTS

Orally administered COS alleviated DSS induced murine experimental colitis: The effect of oral administration of COS on DSS induced murine experimental colitis was assessed using DAI scores, colon lengths and histological examination. The increase of DAI scores, a combined measure of loss of body weight, stool consistency and fecal occult blood, was found starting on day 1 of DSS administration in colitis group and on day 5 in COS treated in colitis group (Fig. 1a) and COS supplementation significantly attenuated the DSS induced increase of DAI scores compared to the corresponding colitis control group ($p<0.05$, $p<0.01$), suggesting orally administered COS could delay the onset of colitis and relieve the cardinal symptoms of UC. In addition, since colon shortening in mice is correlated with the histological changes, e.g., crypt cell damage[28], the administration of COS at the dose of 500 mg kg^{-1} significantly improved DSS induced shortening of colon length ($p<0.01$, Fig. 1b), indicating the disease severity of colitis was reduced by COS. Histological analysis of the distal colon showed that oral administration of COS to normal mice did not influence the structure of colonic mucosa, while COS administration to DSS treated mice significantly ameliorated the typical inflamed signs induced by DSS challenge, including the infiltration of massive inflammatory cells, mucosal ulcerations, extensive epithelial disintegration and loss of goblet cells (Fig.1c). And DSS increased histological activity indices were significantly reduced following COS supplementation ($p<0.01$, Fig.1d). These data suggested orally administered COS was effective in alleviating DSS induced colitis in mice.

Orally administered COS modulated microbial community structure in colonic contents of normal and colitis mice: In order to further investigate the mechanisms linking the oral administration of COS with UC, the total bacterial DNA from

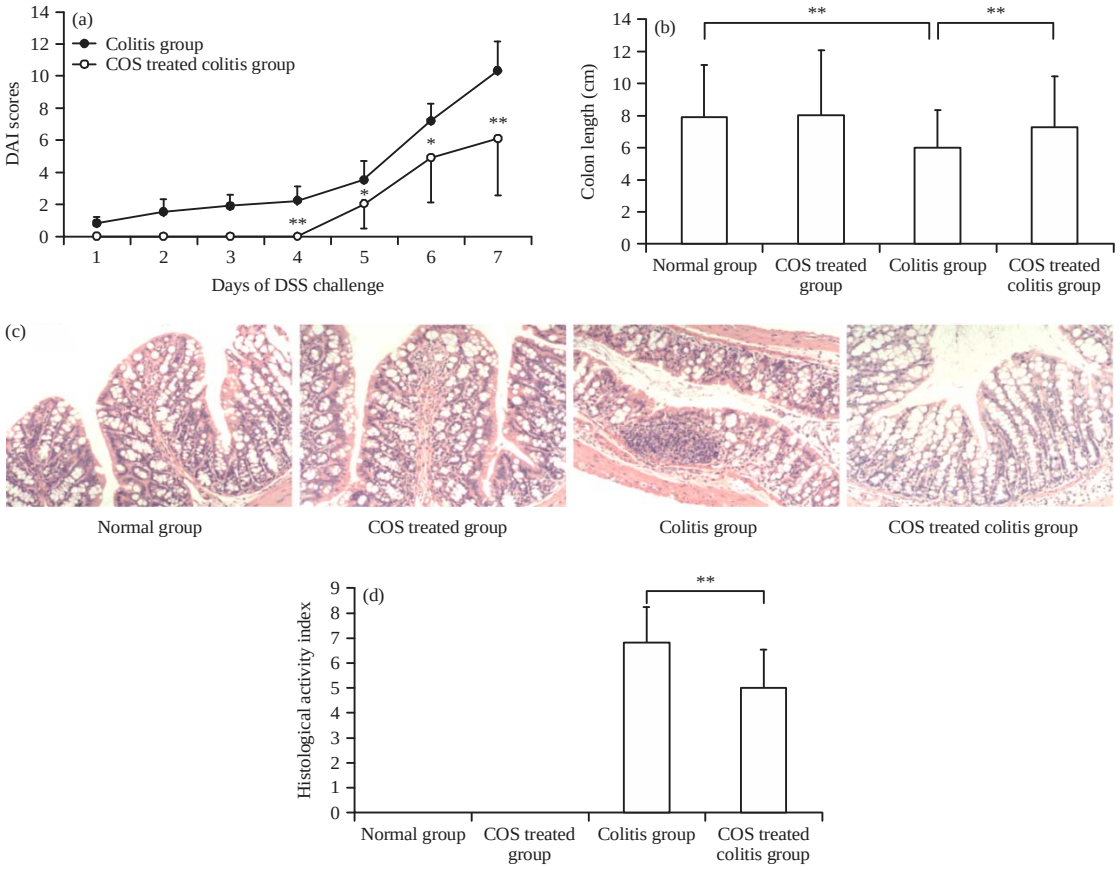

Fig. 1(a-d): Effect of COS on the DAI scores, colon length and colonic inflammation normal and colitis mice, (a) Daily DAI scores from the beginning of DSS challenge to the end of the experimental period, (b) Assessment of colon shortening, (c) Representative HE-stained colon sections and (d) Histology activity index

Values were expressed as the Mean±SD, n = 10, *p<0.05, **p<0.01 as conducted

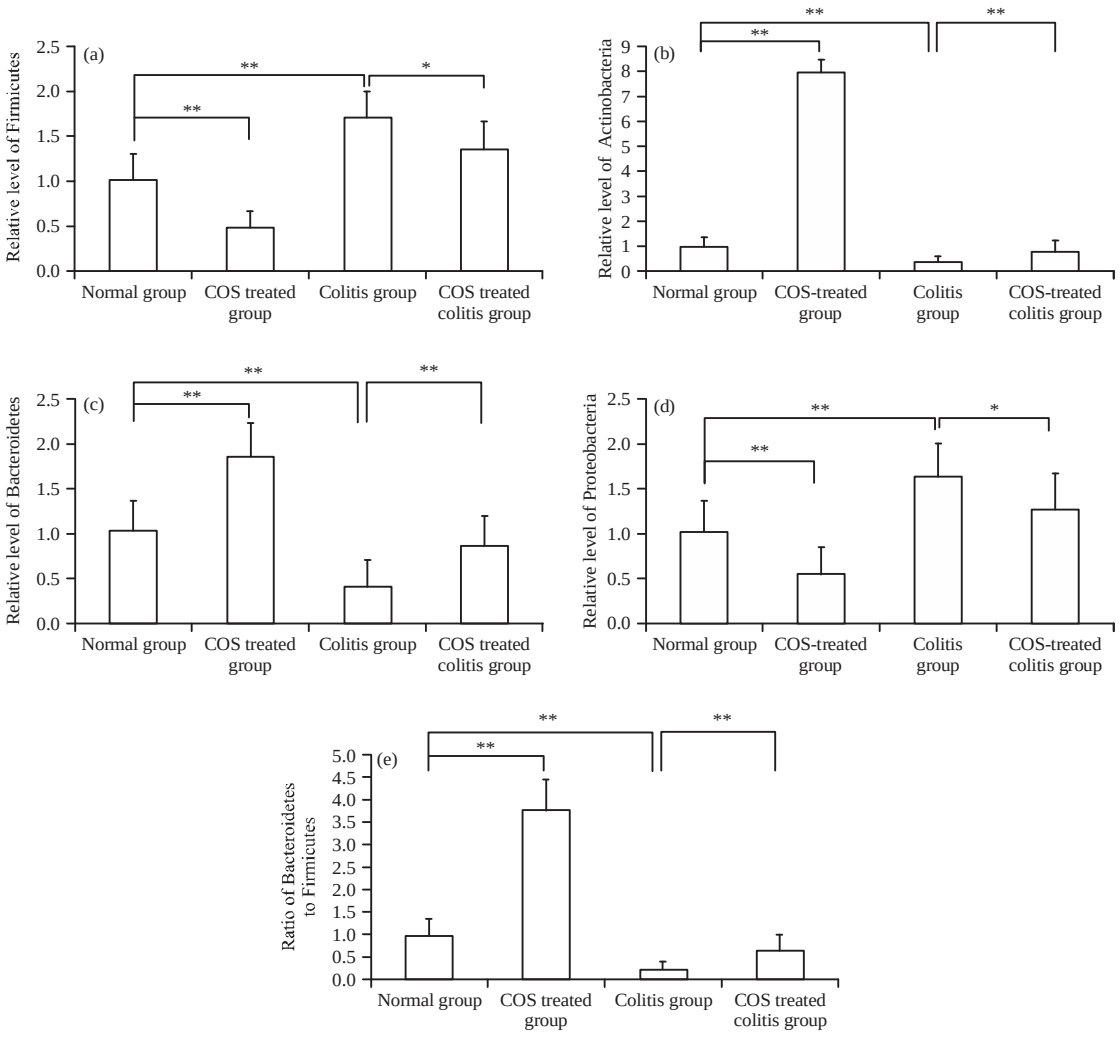

Fig. 2(a-e): COS modulated the microbial community structure at the phylum level in colonic contents of normal and colitis mice
Data are expressed as Mean±SD, n = 10, *p<0.05, **p<0.01 as conducted

colonic content samples was isolated and the quantitative analysis of major bacterial populations via real-time PCR was performed.

At the phylum level (Fig. 2), COS treated group showed the significantly increased gene copy numbers of Bacteroidetes and Actinobacteria phyla in colonic contents, compared with the normal control group ($p<0.01$), that is, 1.81-fold increase in Bacteroidetes and 7.88-fold increase in Actinobacteria were found in mice administrated with COS. In contrast, the proportions of Firmicutes and Proteobacteria phyla were obviously reduced by COS supplementation, 0.47-fold decrease in Firmicutes and 0.55 fold decrease in Proteobacteria with COS supplementation compared with normal mice ($p<0.01$). And the increased ratio of Firmicutes to Bacteroidetes was observed in COS treated group, that is, 3.88 fold increase, in comparison with the normal group ($p<0.01$). In DSS induced colitis group, the populations of Bacteroidetes and Actinobacteria phyla and ratio of Firmicutes to Bacteroidetes were only 40.8, 37.6 and 23.7% of those in normal group, respectively but the levels of Firmicutes and Proteobacteria phyla were significantly higher ($p<0.01$), suggesting the dysbiosis of native gut microbiome in colitis mice. However, this DSS induced dysbiosis was reversed by the administration of COS in colitis mice ($p<0.05$, $p<0.01$).

Further, when differences in the microbiota at the generic level were compared (Fig. 3), results showed that, the intervention with COS in normal mice facilitated the growth and proliferation of probiotics, including *Lactobacillus* and *Bifidobacterium*, in colonic contents: 3.18 fold increase in *Lactobacillus*, 4.97 fold increase *Bifidobacterium* following COS administration compared with normal group ($p<0.01$).

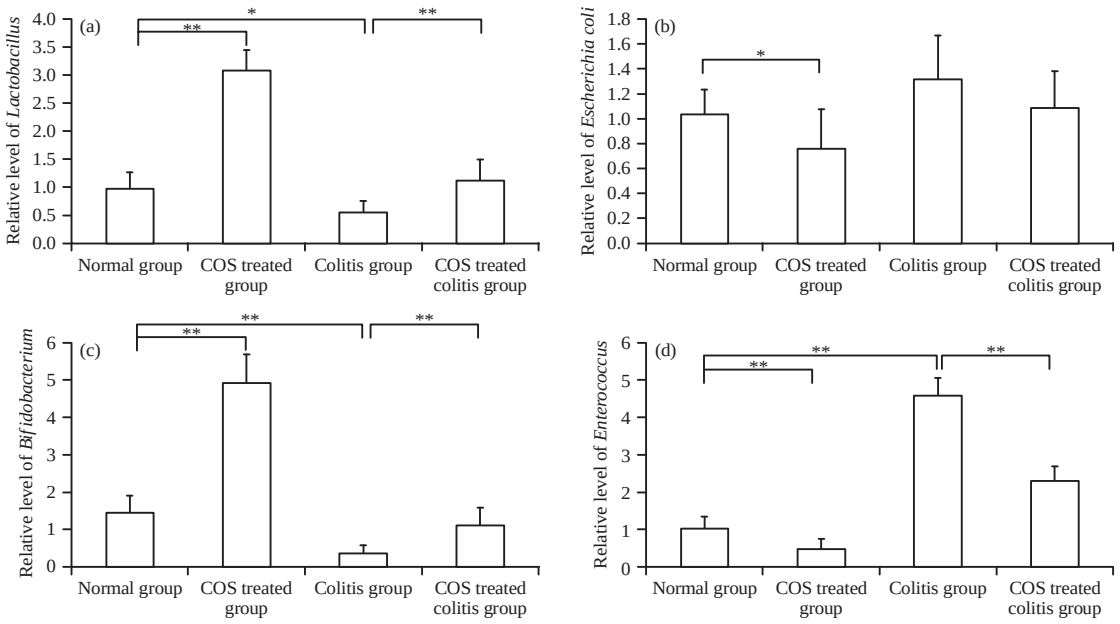

Fig. 3(a-d): COS modulated the microbial community structure at the generic level in colonic contents of normal and colitis mice
Data are expressed as mean±SD, n = 10, *p<0.05, **p<0.01 as conducted

Table 2: Concentrations of short-chain fatty acids (SCFAs) in colonic contents

	SCFA concentration (μmol g^{-1})				
Groups	Total SCFA	Acetate	Propionate	Butyrate	Valerate
Normal group	36.77±5.72	28.67±3.11	5.35±0.23	2.03±0.38	0.72±0.17
COS treated group	45.27±6.18*	35.58±3.94*	5.82±0.10*	3.19±0.42*	0.68±0.37
Colitis group	23.31±5.31**	16.85±4.32**	4.88±0.31*	1.12±0.22**	0.46±0.31
COS treated colitis group	28.49±6.47#	20.33±4.51#	5.14±0.29#	2.59±0.48#	0.43±0.28

Data are expressed as Mean±SD, *p<0.05, **p<0.01 vs normal group, #p<0.05, vs colitis group

But the gene copy numbers of *Enterococcus* spp. and *Escherichia coli* in COS treated group were, respectively 47.5 and 73.8% of those in normal group (p<0.05, p<0.01). In colitis group, DSS challenge induced a significant increase in the abundance of *Enterococcus* reaching 4.6 folds as compared to the normal group (p<0.01). And the levels of *Lactobacillus* and *Bifidobacterium* in colitis mice were only 57.7 and 36.4% of those in normal animals (p<0.01). In COS treated colitis mice, COS administration tended to reduce the DSS induced increase in the counts of *Enterococcus* and improve the decreases of both *Lactobacillus* and *Bifidobacterium* counts compared to the colitis control group (p<0.01).

Orally administered COS altered the concentrations of SCFAs in colonic contents: Based on the above finding that COS showed the ability to modulate microbial community structure, the levels of SCFAs in colonic contents were further examined. As shown in Table 2, DSS challenge significantly decreased the concentrations of total SCFAs, acetate, propionate and butyrate in colonic contents (p<0.05, p<0.01). Importantly, the oral administration of COS at the dose of 500 mg kg^{-1} resulted in a significant increase of the concentrations of total SCFAs, acetate, propionate and butyrate in either normal or DSS treated mice (p<0.05), while the concentration of valerate in colonic contents was unaffected. These data suggested that orally administered COS could increase the SCFA concentration in gut and were likely to protect against the suppression of SCFA production commonly associated with colonic inflammation in UC.

DISCUSSION

Gut flora play an indispensable role in human health, including helping to maintain the normal gut function and contributing to the host defense system of gut, etc.[29]. In human and animal colon, Firmicutes (containing the three main classes Bacilli, Clostridia and Mollicutes) and

Bacteroidetes (including Bacteroides, Prevotella and Xylanibacter) represent the predominant bacterial phyla, which account for 60-80 and 20-30%, respectively. The Bacteroidetes to Firmicutes ratio was a reliable index to assess the composition of gut microflora and important for health and disease. A shift in the abundance of Firmicutes to Bacteroidetes was considered to be responsible for protection against many disorders, such as colorectal cancer, inflammatory bowel disease, disorders of the liver and obesity[30,31]. In addition, the increased ratio of Bacteroidetes to Firmicutes has also been reported in subjects consuming Western high-fat diets[32,33]. Bacteroidetes were the major SCFA-producing bacteria [34]. Among the phylum Firmicutes, the Lactobacillales order (class Bacilli) including the genera *Lactobacillus* and *Enterococcus* were the most dominant. Bacteria of the genus *Enterococcus*, as Gram-positive commensal of the gastrointestinal tract, normally did not cause disease in gut but can be pathogenic when they infect outside of gut, thus had become important nosocomial pathogens capable of causing potentially life-threatening infections in humans[35]. As typical probiotic bacteria, *Lactobacillus*, together with *Bifidobacterium*, the latter of which belong to the phylum Actinobacteria, possess momentous and widely acknowledged immunomodulatory and health-promoting properties and provide means for the prevention and treatment of various allergic, infectious and inflammatory conditions[36]. And Proteobacteria such as *Enterobacteriaceae* (main representative *Escherichia coli*) and *Escherichia* are a family of Gram-negative bacteria that commonly cause infections as pathogens and less found in normal gut[30]. In the present study, the quantitative analysis of major bacterial populations in colonic contents by real-time PCR revealed that, in normal mice, COS administration improved the structure of gut flora by increasing the populations of Bacteroidetes and Actinobacteria phyla, the relative ratio of Bacteroidetes to Firmicutes, as well as the count of common probiotic bacteria including *Lactobacillus* and *Bifidobacterium*. In addition, the proportions of Firmicutes and Proteobacteria phyla, as well as the abundance of *Enterococcus* were decreased in COS treated mice. Results were consistent with the previous *in vitro* studies on the high antibacterial activity of COS on *Escherichia coli*[37-39], *Bacillus subtilis*[38], *Staphylococcus aureus*[38] and *Listeria monocytogenes*[37], as well as their prebiotic effect manifested by the stimulation on the growth of *Bifidobacterium bifidium* and *Lactobacillus* sp.[23].

The effect of COS administration on microbiota profile in colonic contents of DSS induced colitis mice did not differ from that of normal mice. In line with previous studies[19-21], COS treatment of colitis mice, at the dose of 500 mg kg^{-1}, showed a protective effect in acute colitis evidenced by diminished DAI and histological activity indices, as well as a counteraction of the colon shortening. The quantitative analysis of microbiota in colonic contents evidenced a significant decrease in the counts of Bacteroidetes and Actinobacteria phyla, *lactobacilli* and *bifidobacteria* genera and on the contrary, an increase in the abundance of potential pathogenic Firmicutes and Proteobacteria phyla, *Enterococcus* genus in DSS induced colitis mice in comparison with the healthy ones, which agree with the previous studies on the dysbiosis of gut microbiome in colitis patients[5,9] and animals[10,40]. And at the phylum level, Firmicutes and Actinobacteria were found to be negatively correlated with the injury of distal colon induced by DSS administration[40]. But the supplementation with COS tended to effectively correct the dysbiosis associated with DSS induced colitis. Based on the role of dysbiosis in the onset and development of UC, it could be speculated that the effect of COS intake on the colon microbiota may also be one of the mechanisms responsible for COS' protective properties in UC.

Accordingly, the oral administration of COS at the dose of 500 mg kg^{-1} resulted in a significant increase of concentrations of total SCFAs, acetate, propionate and butyrate in normal and colitis mice. The SCFAs including acetate, propionate, butyrate and valerate, etc. were major products derived from the prebiotic fermentation of unabsorbed carbohydrates in colon[41]. The suppression of SCFA production due to dysbiosis of bacterial populations was implicated in the pathogenesis of UC[28,40,41]. The SCFAs contribute to the stabilization of pH in colon considered as a primary supply of energy to the enteric epithelium. Moreover, SCFAs, in particular butyrate, was found to limit the immune cell driven inflammation in colon and induce the apoptosis of mutated epithelial cells[42]. Increased SCFA levels in colon could promote epithelial cell proliferation, stimulate the production of mucin and regulate epithelial cell integrity, which contribute to the maintenance of colonic homeostasis[28,40,41]. As the fermentation products of probiotics, especially Bacteroidetes SCFAs in the colon could be considered as biomarkers of healthy status and be beneficial in improving gastrointestinal health[28]. In line with the previous studies[43,44], DSS administration significantly decreased the concentrations of total SCFAs, acetate, propionate and butyrate which demonstrated that there was some correlation between the concentration of acetate and macroscopic damaged area in the colon[44]. Similarly, Koleva *et al.*[45] also reported that the relative concentration of acetate was negatively correlated to the levels of interleukin-1β and the cecal histology score in

colitis animal. These suggested the close relationship between the levels of SCFAs, in particular acetate and the colonic inflammation. Prebiotics that selectively promote the growth of beneficial microbiota including Bacteroidetes could also induce the changes in SCFA production in patients[28]. In the present study, suggested that, in accordance with the effect on SCFA-producing bacteria, COS could act as prebiotics to increase the levels of SCFAs in normal and colitis mice. In fact, oligosaccharides comprising a short chain of monosaccharide units, have been long considered as substrates for SCFAs[34]. And SCFAs, especially acetate, propionate and butyrate, contributed to intestinal barrier integrity, play a beneficial role in intestinal defense and the maintenance of intestinal homeostasis[28]. Thus, the increased SCFAs levels by COS supplementation may contribute to promote symbiotic gut environment and prevent the development and progression of UC.

CONCLUSION

This study demonstrated that, similar to many oligosaccharides extracted from natural products, COS could function as prebiotics by increasing the levels of beneficial bacteria such as *Lactobacillus* and *Bifidobacterium* and inhibiting the growth of potential pathogens such as *Enterococcus*. In addition, oral intake of COS were found to enhance the colonic concentrations of SCFAs, the dominating fermentation end-products of bacteria in the large bowel having abilities to support the transport processes, energy metabolism, cellular growth and differentiation of colonocytes. These data suggested that COS administration might have beneficial effects on the health of the intestinal tract. Furthermore, based on the mouse experimental colitis following DSS challenge, a well accepted colonic inflammation model resembling human UC, this study demonstrated that COS possessed the preventive effect on the development of colitis and most importantly, tended to protect mice from dysbiosis of native gut microbiome and against the suppression of SCFA production, which might be a potential mechanism for their anti colitis effect.

SIGNIFICANCE STATEMENT

This study discover the ability of chitooligosaccharides (COS) to modulate the colon microbial composition in normal and dextran sulfate sodium (DSS) induced colitis mice, that can be beneficial to confirm the prebiotic property of COS *in vivo* and further clarify the mechanisms of their anti colitis effect. This study help the researchers to uncover the critical areas of the exact anti colitis mechanism of COS.

ACKNOWLEDGMENT

All authors of the manuscript acknowledge and thank their respective Universities and Institutes. This study was supported by the National Natural Science Foundation of China (81602108).

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