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ORIGINAL RESEARCH

Selenium Yeast Alleviates Dextran Sulfate Sodium-Induced Chronic Colitis in Mice by Reducing Proinflammatory Cytokines and Regulating the Gut Microbiota and Their Metabolites

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Background: Inflammatory bowel disease (IBD) is a chronic recurrent gastrointestinal inflammatory disease. Selenium has been reported to have therapeutic potential in IBD. Selenium yeast is a common selenium supplement that is convenient to access. This study explored the effect of selenium yeast on dextran sulfate sodium- (DSS-)induced chronic colitis in mice.
Methods: Mice were randomly divided into four groups: the control group, selenium yeast group, chronic colitis group, and chronic colitis+selenium yeast group (n=6). Mice were killed on the 26th day. The disease activity index (DAI) score and histological damage score were calculated. Cytokines, serum selenium, colonic tissue selenium, gut microbiota and their metabolites short-chain fatty acids (SCFAs) were evaluated.
Results: Selenium yeast lowered IL-1β, IL-6, TNF-α, IL-17A, IL-22 and IFN-γ ($P<0.05$). In addition, selenium yeast significantly elevated *Turicibacter, Bifidobacterium, Allobaculum, Prevotella, Halomonas, Adlercreutzia* ($P<0.05$), and butyric acid ($P<0.05$).
Conclusion: Selenium yeast could improve DSS-induced chronic colitis in mice by regulating cytokines, gut microbiota and their metabolites.
Keywords: selenium yeast, chronic colitis, gut microbiota, metabolism

Introduction

Inflammatory bowel disease (IBD) is a chronic recurrent gastrointestinal inflammatory disease that mainly includes ulcerative colitis (UC) and Crohn's disease (CD).[1] The pathogenesis of IBD is not fully understood. Genes, immunity, diet, environment, gut microbiota and their metabolites are all related to the pathogenesis of IBD.[2–4]

Cytokines play an important role in the occurrence and persistence of IBD inflammation.[5,6] Drugs targeting certain cytokines, such as infliximab, the first tumor necrosis factor-(TNF-)αblocker, can alleviate IBD.[7]

Changes in the gut microbiota are also crucial for IBD. Some gut microbiota producing butyric acid in IBD patients decrease, such as *Clostridium clusters IV* and *XIVa* and *Faecalibacterium prausnitzii*.[8] The gut microbiota could be used to distinguish CD from non-CD. Pascal et al reported that an unknown *Peptostreptococcaceae, Faecalibacterium, Anaerostipes, Methanobrevibacter*, and an unknown *Christensenellaceae*, were abundant in healthy people and UC patients. *Fusobacterium* and *Escherichia* were abundant in CD patients. *Collinsella* was abundant in UC patients.[9] The change in gut microbiota can also be used to predict whether anti-TNF-α is effective for IBD patients. It was reported that patients with a high abundance of *F. prausnitzii* and *Ruminococcus* and a low abundance of *Methanobrevibacter smithii* have a good response to anti-TNF-α

treatment.[10] In addition, gut microbiota dysbiosis exists not only in patients themselves but also in brothers and sisters of Crohn's disease patients.[11] Metabolites of the gut microbiota, such as short-chain fatty acids (SCFAs), also play an important role in maintaining intestinal homeostasis. Studies have found that SCFAs can regulate the homeostasis of T cells in the colon and promote the production of IL-22 by innate lymphocytes and CD4+ T cells to prevent intestinal inflammation and maintain intestinal homeostasis.[12,13]

Selenium is an important trace element in the human body that has anti-inflammatory and antioxidant effects.[14–16] It can be divided into inorganic selenium and organic selenium. Inorganic selenium mainly includes selenite and selenate, and organic selenium mainly includes selenomethionine and selenocysteine.[17] Selenium can alleviate inflammation-mediated by cyclooxygenase (COX), which plays an important role in IBD.[18] Selenium can also relieve intestinal inflammation by inhibiting the excessive immune response induced by Th1 cells.[19] In addition, selenium can affect the composition and colonization of gut microbiota.[20] Sodium selenite has been reported to alleviate dextran sulfate sodium- (DSS-) induced chronic colitis in mice.[21] Selenium deficiency in IBD patients has been reported. A Japanese study found that 10.9% of children with IBD have selenium deficiency, and there is more selenium deficiency in CD patients than in UC patients.[22] A Korean study also found that 30.9% of patients with IBD have selenium deficiency.[23] Above all, selenium may play an important role in intestinal homeostasis.

Compared with inorganic selenium, organic selenium is better absorbed.[24] Selenium yeast is produced by fermenting yeast in a selenium-rich environment to combine selenium with components in yeast to form organic selenium,[25] in which the main form of selenium is selenomethionine.[26] An animal experiment found that the specific bioavailability of selenium yeast was higher than that of sodium selenite.[27] Therefore, we explored the effect of selenium yeast on mouse chronic colitis induced by DSS and its possible mechanism.

Materials and Methods
Animal and Ethical Matters
Twenty-four 8-week-old specific pathogen-free C57BL/6 male mice weighing 24±2 g were purchased from Liaoning Changsheng Biology and bred under specific pathogen-free conditions (a 12 h light/12 h dark-light regimen, temperature 23±2 °C, humidity 50–60%). Selenium yeast (selenium>2000 ppm) was purchased from Shanghai Yuanye Biotechnology Co., Ltd. The research protocol was approved by the Animal Ethics Committee and Animal Care Committee of China Medical University. Ethical batch number: cmu2021321. The research followed Laboratory animals—Guideline ethical review of animal welfare (GB/T 35892-2018).

Experimental Design
Twenty-four mice were divided randomly into four groups: 6 in the control group (Group A), 6 in the selenium yeast group (Group B), 6 in the chronic colitis group (Group C), and 6 in the chronic colitis + selenium yeast group (Group D). The control group was given tap water and a normal diet. The selenium yeast group was given tap water and a normal diet, with selenium yeast (100 mg/kg) gavage once a day. The chronic colitis group was induced colitis by 1.5% DSS and given a normal diet. The chronic colitis + selenium yeast group was induced colitis by 1.5% DSS and given a normal diet with selenium yeast (100 mg/kg) gavage once a day. Weight and disease activity index were recorded every day.

Induction of Chronic Colitis by DSS
Oral administration of 1.5% DSS (molecular mass 36–50 kDa; MP Biomedicals, Solon, OH,

United States) was used to induce colitis on Days 0–5, 10–15, and 20–25 d and tap water on the other days.[28] The mice were sacrificed on the 26th day.

Disease Activity Index
The severity of colitis in mice was assessed by the disease activity index (DAI), which consists of the percentage of weight loss (0–4 points), stool consistency (0–4 points), and intestinal bleeding (0–4 points),[28] as shown in Table 1.

Table 1 Disease Activity Index (DAI) Score

Score	Weight Loss (%)	Stool Property	Bleeding
0	0	Normal	Normal
1	1–5		
2	>5–10	Loose	Fecal occult blood
3	>10–15		
4	>15	Diarrhea	Bleeding

Histological Injury Score

After the mice were sacrificed, we used 4% paraformaldehyde to fix colon tissues and embedded the colon tissues in paraffin. The colon tissues were stained with hematoxylin and eosin after being cut into 4-μm sections and scored for histological damage. Two pathologists assessed histological scores independently in a blinded fashion. The sum of scores of inflammation severity, degree of mucosal damage, percentage of crypt damage, and pathological change range were calculated. None, mild, moderate, or severe inflammation was quantified as the percentage involvement by inflammation (none, 0–33%, 33–67%, 67–100%). The depth of inflammation (none, mucous layer, submucosa, muscularis, and serosa) represented mucosal damage, as shown in Table 2.[28]

Cell Preparation, Culture, and Activation

The large intestine of each mouse was cut into 1–2 mm pieces. The pieces were stirred in PBS containing 3 mmol/L EDTA twice (15 min each time) and twice in RPMI 1640 (HyClone) containing 1 mmol/L EGTA (20 min each time) at 37 °C to eliminate the epithelium. The remaining pieces were stirred at 37 °C for 90 min in RPMI 1640 (HyClone) containing 20% fetal bovine serum, 100 U/mL collagenase (C2139; Sigma–Aldrich Corp., St. Louis, MO, United States) and 5 U/mL DNase1 (Sigma–Aldrich Corp). The suspensions were centrifuged, and the pellets were cleaned. Lamina propria lymphocytes (LPLs) were isolated from lamina propria (LP) cell preparations by centrifugation with a 45–66.6% discontinuous Percoll (Solarbio) gradient at 2500 rpm for 20 min.

In an atmosphere containing 5% CO_2, 96-well plates coated with anti-CD3 (10 μg/mL e-Bioscience, San Diego, CA, United States) and soluble anti-CD28 (1 μg/mL, e-Bioscience) monoclonal antibodies (mAbs) were used to culture LPLs (1×10^5/well in 0.2 mL of RPMI 1640 medium containing 10% fetal bovine serum, 1% penicillin, and 1% streptomycin) at 37 °C for 48 h. The supernatants were collected, and the cytokines and myeloperoxidase (MPO) were assessed by enzyme-linked immunosorbent assay after 48 h.[28]

Enzyme-Linked Immunosorbent Assay

The cell culture supernatants were collected after centrifugation at 1000 rpm for 10 min according to the manufacturer's instructions. Mouse immunoassay kits (R&D Systems Inc., Minneapolis, MN, United States) were used to measure cytokine concentrations. MPO kits were used to measure the activity of MPO in the colon tissue. The levels of IL-6, IL-1β, TNF-α and MPO were measured in the supernatants. The levels of IFN-γ, IL-17A, IL-21, IL-22 and IL-10 were measured in the supernatants with or without anti-CD28/anti-CD3 mAbs stimulation.[28]

Table 2 Histological Injury Score

Grade	0	1	2	3	4
Inflammation	None	Mild	Moderate	Severe	–
Mucosal damage	None	Mucous layer	Submucosa	Muscularis and serosa	–
Crypt damage	None	1/3	2/3	100%	100% with epithelium loss
Pathological change range	None	0%-25%	26%-50%	51%-75%	76%-100%

Determination of Selenium in Serum and Colon Tissue

Fluorescence atomic absorption spectrometry was used to determine the selenium content in colon tissue. The concentrations of serum selenium were detected in duplicate by inductively coupled plasma–mass spectrometry (ICP–MS, Perkin-Elmer SCIEX ElAN 6000, US).[21]

DNA Extraction and Amplification

Shanghai Personal Biotechnology Co., Ltd. (Shanghai, China) performed DNA extraction, sequencing and analysis. The sample microbial DNA was extracted using the OMEGA Soil DNA Kit (M5635-02) (Omega Bio Tek, Norcross, GA, USA) according to the instructions. The total DNA quantity and quality were tested by a NanoDrop NC2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and 0.8% agarose gel electrophoresis respectively. The forward primer 338F (5'-ACTCCTACGGGAGGCAGCA-3') and the reverse primer 806R (5'-GGACTACHVGGGTWTCTAAT-3') were used to perform PCR amplification of the bacterial 16S rRNA genes V3–V4 region. PCR amplicons were purified by Vazyme VAHTSTM DNA Clean Beads (Vazyme, Nanjing, China) and quantified by the Quantit PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA).

DNA Sequencing and Analysis

QIIME2 2019.4[29] was used to perform microbiome bioinformatics with slight modification according to the official tutorials (https://docs.qiime2.org/2019.4/tutorials/). In brief, raw sequence data were demultiplexed using the demux plugin followed by primer cutting with the cutadapt plugin.[30] Sequences were quality filtered, denoised, merged and chimera removed using the DADA2 plugin then.[31] Taxonomy was assigned to non-singleton amplicon sequence variants (ASVs) using the classify-sklearn naïve Bayes taxonomy classifier in the feature-classifier plugin[32] against SILVA Release 132.

Sequence data analyses were mainly performed using QIIME2 and R packages (v3.2.0). ASV-level alpha diversity indices, such as observed species, Chao 1 richness estimator, Shannon diversity index, Faith's PD, Simpson index, Good's coverage and Pielou's evenness were calculated using the ASV table in QIIME2 and visualized as box plots. Beta diversity analysis was performed to investigate the structural variation in microbial communities across samples using Bray–Curtis metrics[33] and visualized via principal coordinate analysis (PCoA). The significance of differentiation of microbiota structure among groups was assessed by permutational multivariate analysis of variance (PERMANOVA)[34] using QIIME2. Linear discriminant analysis effect size (LEfSe) was performed to detect differentially abundant taxa across groups using the default parameters.[35] The correlation analysis was performed by the genescloud tools, a free online platform for data analysis (https://www.genescloud.cn).

Determination of SCFAs

The large intestinal content samples were placed into 2 mL EP tubes, extracted with 0.5 mL of distilled water, and vortexed for 10s. The samples were incubated in ice water, homogenized in a ball mill at 45 Hz for 4 min and then treated with ultrasound for 5 min. The samples were centrifuged at 4 °C at 12,000 rpm for 15 min. Then, 0.3 mL of supernatant was transferred into fresh 2 mL EP tubes, and the previous steps were repeated. A total of 0.8 mL of supernatant was collected. Then, 0.1 mL of 50% H_2SO_4 and 0.5 mL of 2-methylvaleric acid were added to the supernatant. The mixture was vortexed for 1 minute and centrifuged at 12,000 rpm for 15 min at 4 °C. Agilent 7890 gas chromatography coupled with an Agilent 7000D mass spectrometer (Agilent Technologies, Wilmington, DE, USA) was used for analysis. The system used an HP-FFAP capillary column. In split mode (5:1), a 1 μL aliquot of the analyte was injected. Helium was the carrier gas. The purge flow rate at the front inlet was 3 mL/min, and the gas flow rate was 1 mL/min. The initial temperature was 80 °C for 1 minute, raised to 150 °C at a speed of 5 °C/min, and then raised to 230 °C at a speed of 40 °C/min kept for 12 minutes. The temperatures of the injection port, transfer line, quadrupole and ion source were 240°C, 240°C, 150°C and 230°C, respectively. In the electron impact mode, the energy was −70 eV. After a solvent delay of 5 minutes, the mass spectrum data were recorded in full scan mode with a m/z range of 33–200.

Data Analysis

The data are expressed as the mean ± standard error. The Shapiro–Wilk test was used for normality analysis. If the data conformed to normal distribution and homogeneity of variance, analysis of variance or *t*-test was used. If the data conformed to normal distribution and heterogeneity of variance, the Welch test or *t*- test was used. If the data did not conform to normal distribution, nonparametric test was used. DAI score was analyzed by generalized estimating equation (GEE). The difference was statistically significant when $P<0.05$. For SCFAs, $P<0.05$ and fold change < 0.5 or>2 indicated statistical significance. SPSS version 22.0 (SPSS, Inc., Chicago, IL, United States) was used for data analysis, and GraphPad Prism 6.0 (GraphPad Software, Inc., La Jolla, CA, United States) was used for drawing.

Result

Selenium Yeast Could Alleviate DSS-Induced Chronic Colitis in Mice

The effect of selenium yeast on DSS-induced chronic colitis in mice was evaluated by comparing the difference between the chronic colitis group and the chronic colitis+selenium yeast group. The DAI score of the chronic colitis+selenium yeast group was significantly lower than that of the chronic colitis group on the 16th, 17th, 18th, 19th, 24th and 25th days ($P<0.05$). The histological injury score in the chronic colitis+selenium yeast group was significantly lower than that in the chronic colitis group ($P<0.05$) (Figure 1A–C).

Selenium Yeast Can Regulate Cytokine, MPO and Selenium Concentrations

The concentrations of cytokines detected by ELISA showed that IL-1β, IL-6, and TNF-α increased significantly in the chronic colitis group ($P<0.001$). Selenium yeast could significantly decrease the concentration of these cytokines ($P<0.001$). Compared with the control group, the activity of MPO in the chronic colitis group increased significantly, and selenium yeast significantly reduced the activity of MPO ($P<0.05$). Compared with the control group, in the supernatant without CD3/CD28 stimulation, IL-10, IL-17A, IL-21, IFN-γ, and IL-22 significantly increased in the chronic colitis group ($P<0.01$). Selenium yeast significantly decreased IFN-γ, IL-22 and IL-17A ($P<0.05$). Compared with the control group, in the supernatant stimulated by CD3/CD28, IL-10, IL-17A, IL-21, IFN-γ, and IL-22 increased significantly in the chronic colitis group ($P<0.01$). Selenium yeast significantly decreased IFN-γ, IL-22 and IL-17A ($P<0.01$). Compared with the control group, the serum selenium and colon tissue selenium in the chronic colitis group decreased significantly ($P<0.01$), while selenium yeast significantly elevated the serum selenium ($P<0.01$) (Figure 2A–D).

Selenium Yeast Could Regulate Gut Microbiota

The relative abundance of gut microbiota in different groups was different at the phylum level (Figure 3A). There was a significant difference in Faith's PD between the control group and the chronic colitis group ($P<0.05$), and there was no significant difference in the Chao 1 index, Shannon index, Good's coverage index, Simpson index, Pielou's evenness index, and observed species ($P>0.05$) (Figure 3B). There was a significant difference in β diversity between the control group and the chronic colitis group ($P<0.05$). There was a significant difference in β diversity between the selenium yeast +chronic colitis group and the chronic colitis group ($P<0.05$) (Figure 3C and D).

At the phylum level, compared with the control group, *Firmicutes* in the chronic colitis group decreased significantly ($P<0.05$). Compared with the chronic colitis group, *Firmicutes* in the selenium yeast+chronic colitis group increased significantly ($P<0.05$). At the genus level, compared with the control group, *Lactobacillus* in the chronic colitis group decreased significantly ($P<0.05$), while *Bacteroides, Akkermansia, Turicibacter*, and *Sutterella* increased significantly ($P<0.05$). Compared with the chronic colitis group, *Turicibacter, Bifidobacterium, Allobaculum, Prevotella, Halomonas*, and *Adlercreutzia* increased significantly in the selenium yeast+chronic colitis group ($P<0.05$) (Figure 4A–D).

Selenium Yeast Could Regulate SCFAs, Metabolites of the Gut Microbiota

Selenium yeast could regulate SCFAs. Compared with the control group, butyric acid in the chronic colitis group decreased significantly ($P<0.05$), while selenium yeast significantly increased the amount of butyric acid in the intestine of mice with DSS-induced chronic colitis ($P<0.05$) (Figure 5).
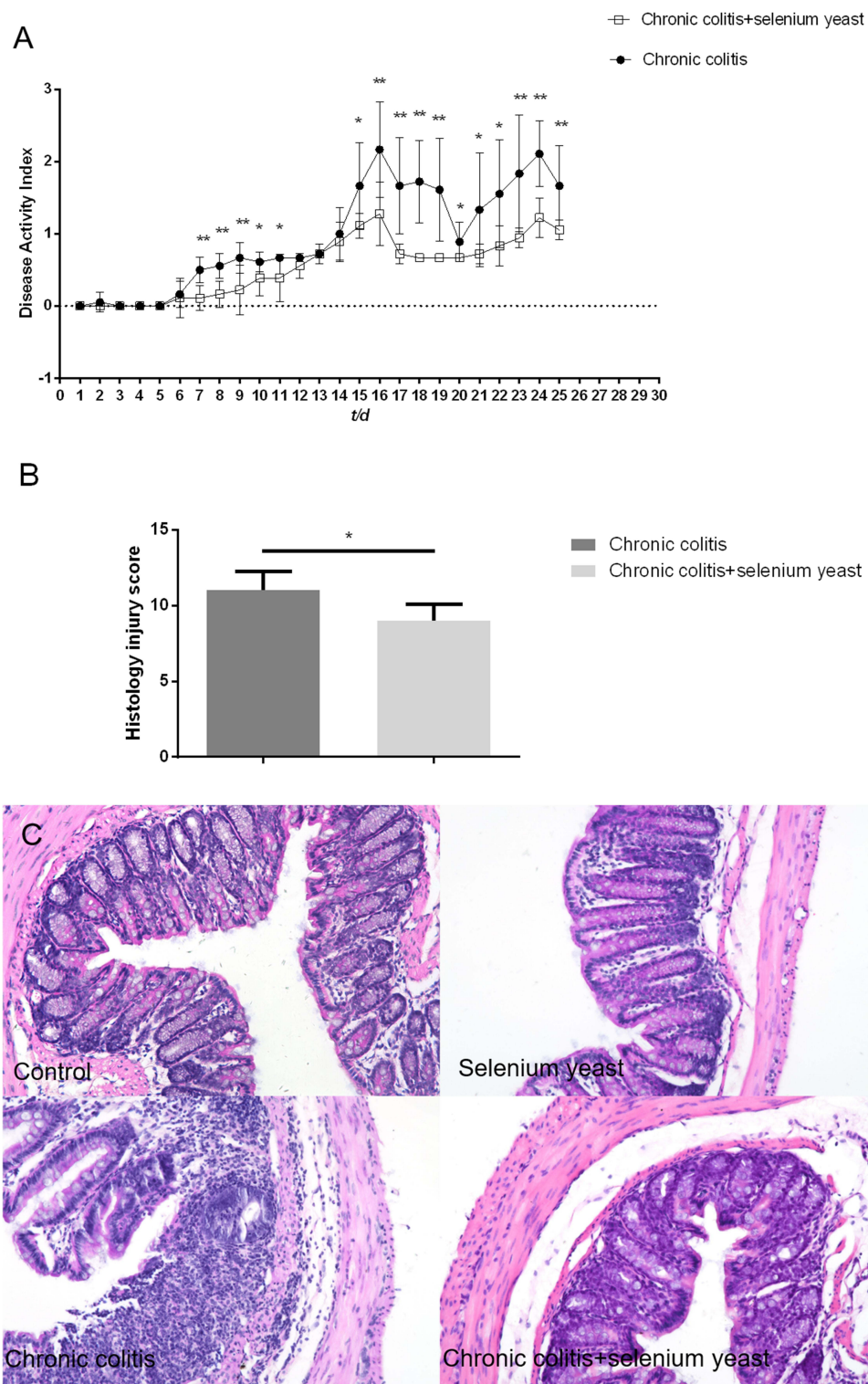
Figure 1 (**A**) Chronic colitis group and chronic colitis + selenium yeast group DAI scores (n = 6); (**B**) Histological injury scores between the chronic colitis group and the chronic colitis + selenium yeast group (n = 6); (**C**) H&E staining of colon tissue of four groups (×200): intestinal tissue revealed the infiltration of inflammatory cells and disruption of crypts in the chronic colitis group. Data are expressed as the mean ± standard error (*$P < 0.05$; **$P < 0.01$).

Correlation Analysis

To evaluate whether there are correlations between gut microbiota, cytokines and SCFAs, we conducted a correlation analysis. At the phylum level, *Firmicutes* was negatively correlated with INF-γ, IL-10, IL-17A, IL-21, IL-22, IL-1β, IL-6, TNF-α, and
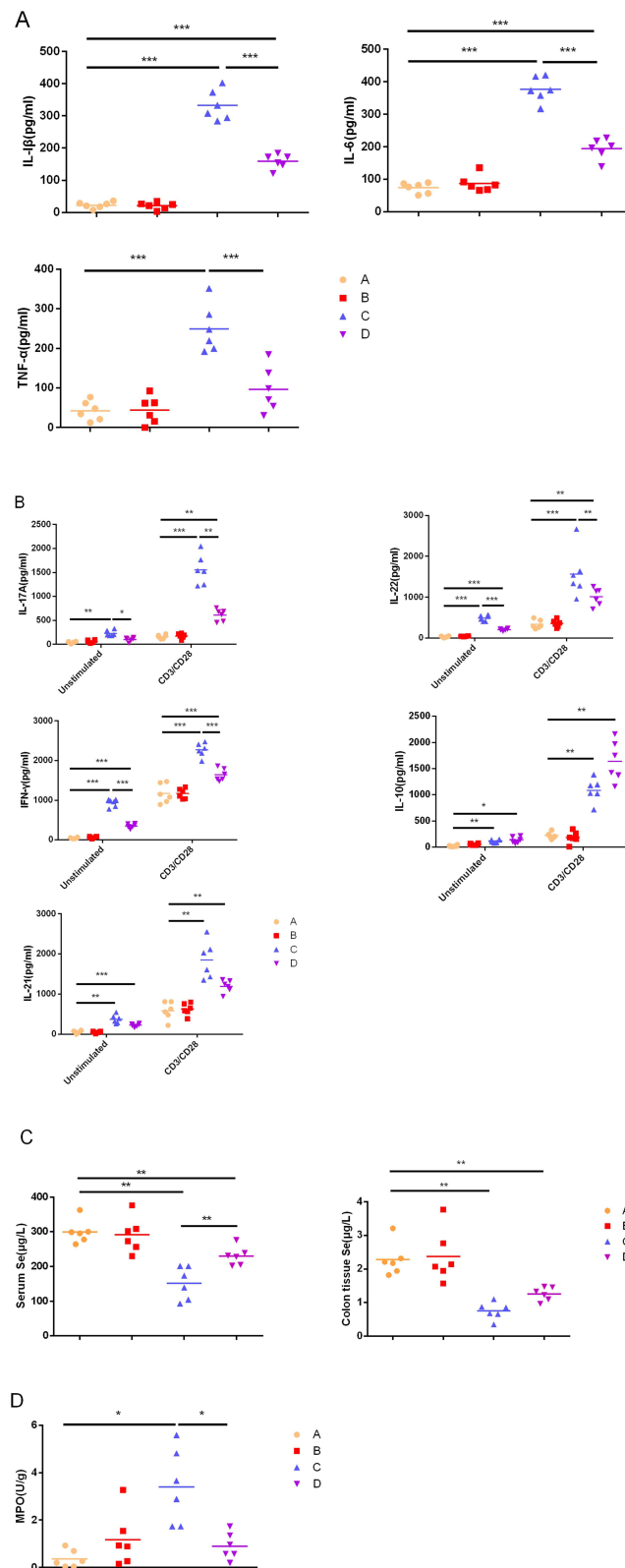
Figure 2 Cytokine concentrations produced by LPL cells, Se concentrations in serum and colon tissue and MPO activity. (**A**) Unstimulated cells; (**B**) LPL cells with or without anti-CD3 and anti-CD28 mAbs; (**C**) Se concentrations in serum and colon tissue. (**D**) MPO activity in colon tissue. The values are expressed as the mean. (*P<0.05; **P < 0.01; ***P < 0.001) (n = 6).
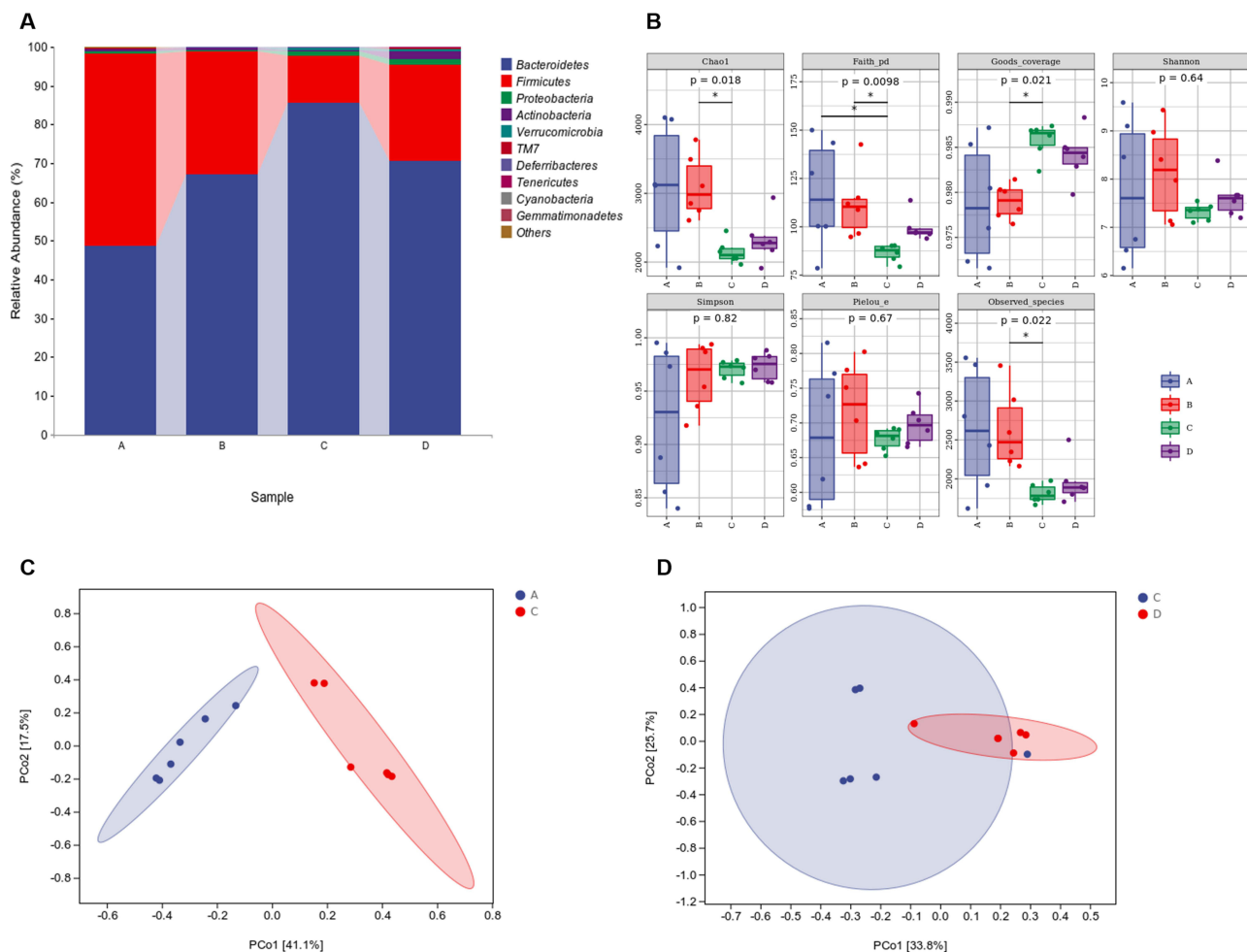
Figure 3 The relative abundance of gut microbiota at the phylum level, α diversity index and β diversity index. (**A**). The relative abundance of gut microbiota in the four groups at the phylum level. (**B**). There was a significant difference in Faith's PD between the control group and the chronic colitis group (*$P<0.05$), and there was no significant difference in the Chao 1 index, Shannon index, Good's coverage index, Simpson index, Pielou's evenness index, and observed species ($P>0.05$). (**C**). The difference in β diversity between the control group (Group A) and the chronic colitis group (Group C) was statistically significant ($P<0.05$). (**D**). The difference in β diversity between the chronic colitis group (Group C) and the chronic colitis + selenium yeast group (Group D) was statistically significant ($P<0.05$).

MPO. At the genus level, *Akkermansia* was positively correlated with IFN-γ, IL-10, IL-21, IL-1β and IL-6. *Bacteroides* and *Turicibacter* were positively correlated with INF-γ, IL-10, IL-17A, IL-21, IL-22, IL-1β, IL-6, and TNF-α. Butyric acid was negatively correlated with INF-γ, IL-17A, IL-21, IL-22, IL-1β, IL-6, TNF-α, and MPO. There were negative correlations between *Bacteroides* and acidic acid, N-valid acid, propionic acid, and butyric acid, while there were negative correlations between *Turicibacter* and acidic acid and N-valid acid, as shown in Figure 6.

Discussion

The protective effect of inorganic selenium, such as sodium selenite, on acute DSS-induced colitis has been reported.[21] Selenium-containing amino acids could alleviate DSS-induced IBD in mice by reducing oxidative stress and the inflammatory response.[36] Organic selenium can be better absorbed and utilized than inorganic selenium.[24,27] Our research found that selenium yeast has a therapeutic effect on DSS-induced chronic colitis by increasing anti-inflammatory factors, reducing proinflammatory factors, and regulating gut microbiota.

The phylum *Firmicutes* in the intestine of patients with IBD decreased.[37] In our study, we found that the phylum *Firmicutes* decreased in mice with DSS-induced chronic colitis, while selenium yeast increased intestinal *Firmicutes*. In addition, one study found that the abundance of *Akkermansia* increased in mice with DSS-induced acute colitis,[38] which is consistent with our findings. However, a systematic review found that the abundance of *Akkermansia* in UC patients
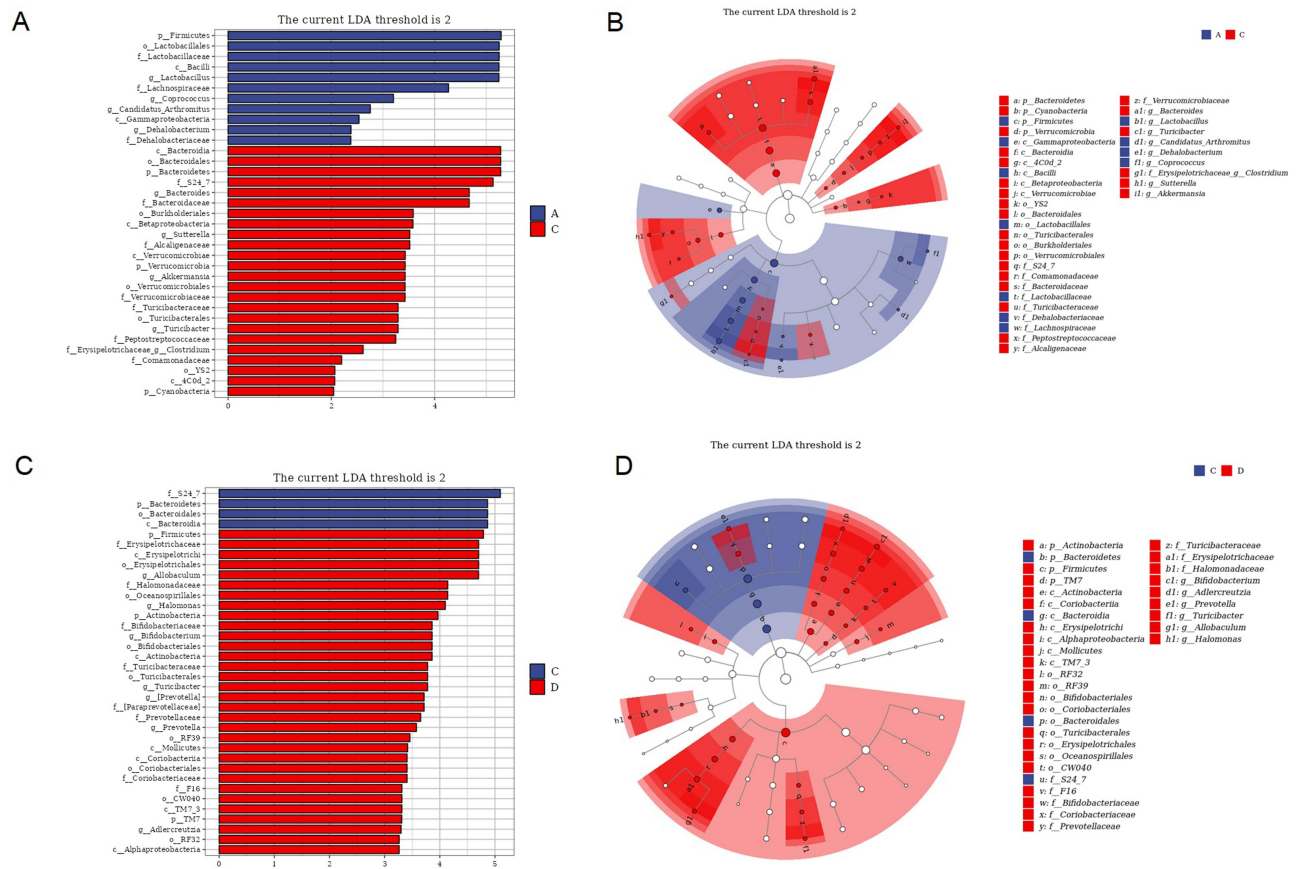
Figure 4 Selenium yeast changes the gut microbiota. Chronic colitis group (Group C); (**A**) Marker bacteria (LDA score (log 10) > 2) between the control group (Group A) and the chronic colitis group (Group C); (**B**) A LEfSe cladogram shows the dominant species in the control group (Group A) and the chronic colitis group (Group C); (**C**) Marker bacteria (LDA score (log10) > 2) between the chronic colitis group (Group C) and the chronic colitis + selenium yeast group (Group D); (**D**) A LEfSe cladogram shows the dominant species of the chronic colitis group (Group C) and the chronic colitis + selenium yeast group (Group D).
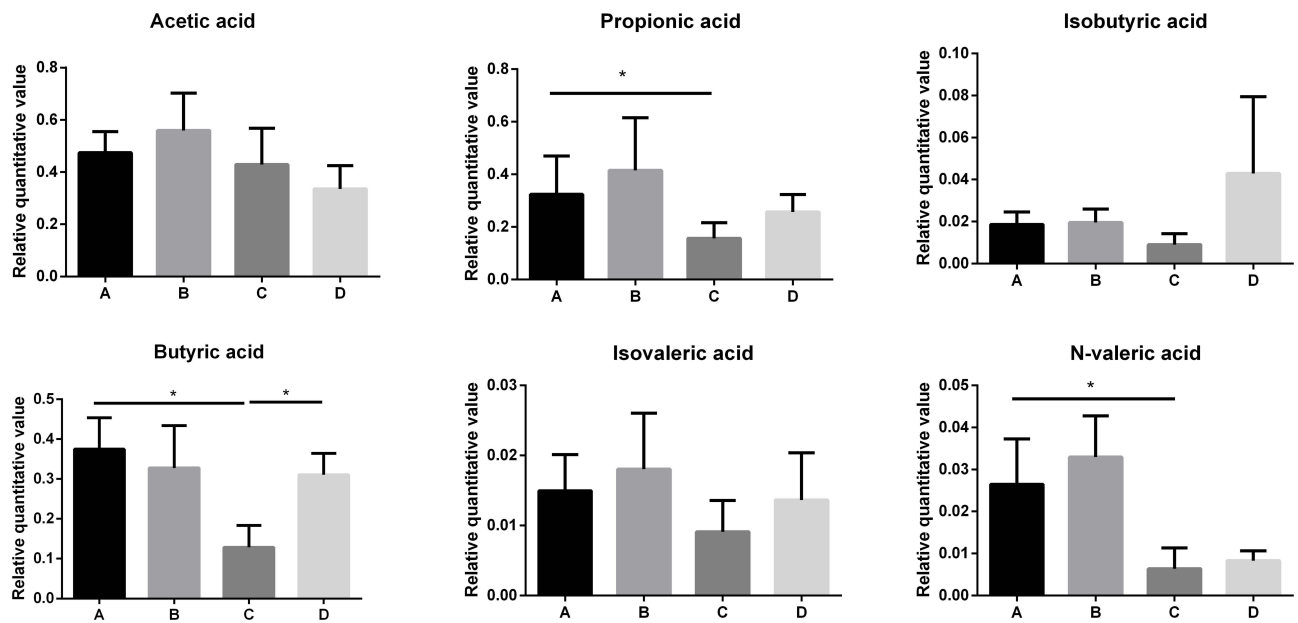

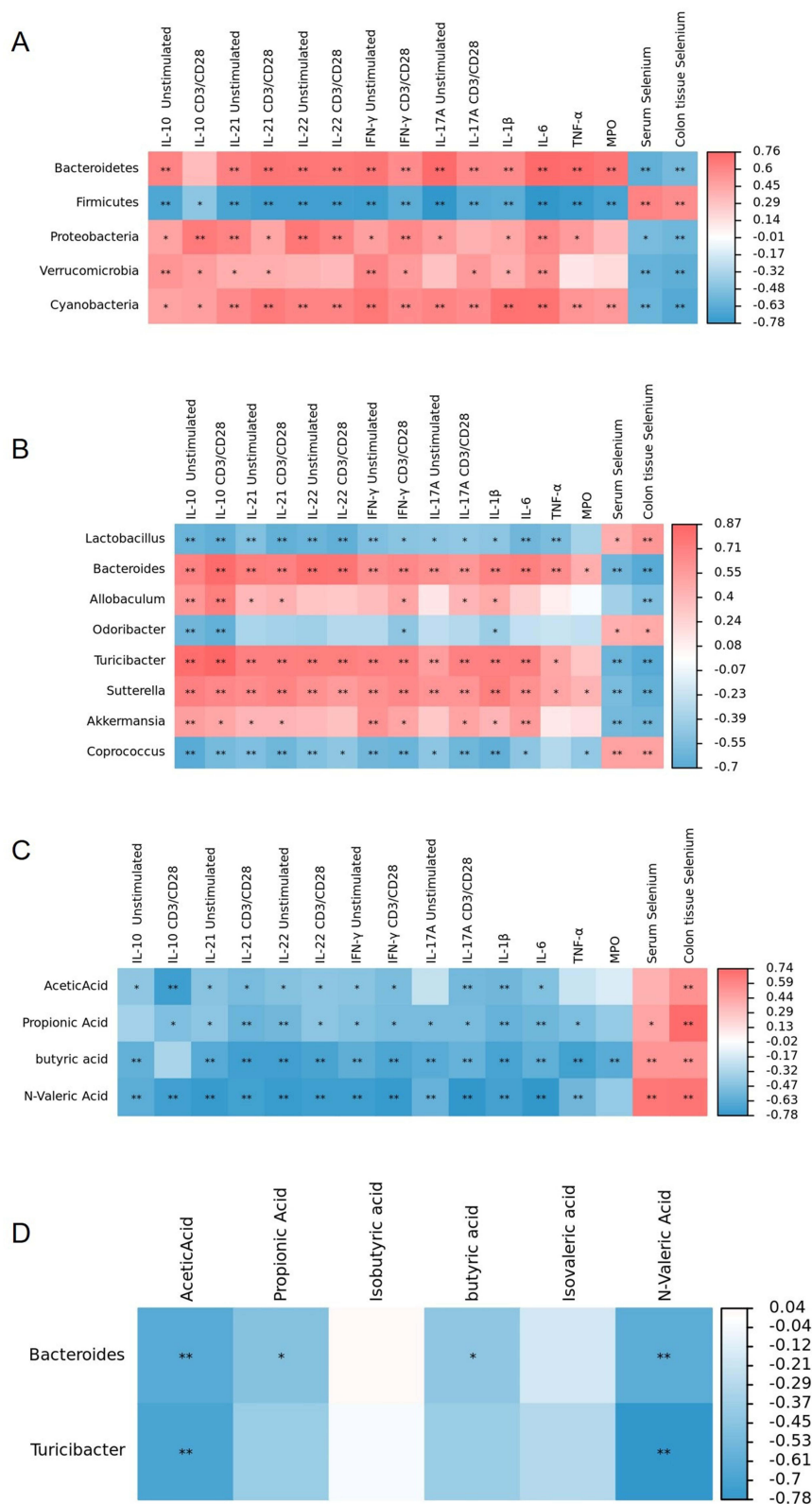**Figure 5** Relative quantitative value of SCFAs between different groups (*P<0.05).

Figure 6 The correlation heatmap shows the correlation among gut microbiota, SCFAs, cytokines, MPO, serum and colon tissue selenium concentrations. Red shows a positive correlation, and blue shows a negative correlation. (**A**) Correlation heatmap between gut microbiota at the phylum level and cytokines, MPO, serum and colonic tissue selenium concentration; (**B**) Correlation heatmap between gut microbiota at the genus level and cytokines, MPO, serum and colon tissue selenium concentration; (**C**) Correlation heatmap between SCFAs and cytokines, MPO, serum and colon tissue selenium concentration; (**D**) Correlation heatmap between gut microbiota at the genus level and SCFAs. (*$P<0.05$; **$P<0.01$).

decreased,[39] and some studies showed that polyphenol-rich cranberry extract and chlorogenic acid could alleviate colitis by increasing *Akkermansia* abundance.[40,41] Another study reported that *Akkermansia* is positively correlated with IL-10,[42] and IL-10 is an important anti-inflammatory factor.[43] Mice with DSS-induced acute colitis had an increase in the abundance of *Turicibacter*.[44] Our research also found that the abundance of *Turicibacter* increased in mice with DSS-induced chronic colitis. Interestingly, our research also found that selenium yeast can increase the abundance of *Turicibacter*. It has been reported that selenium has a regulatory effect on gut microbiota and can increase the relative abundance of *Turicibacter*, which has an anti-inflammatory effect.[45] However, Rossi et al reported a decrease in the abundance of *Turicibacter* in the intestine of dogs with IBD.[46] These findings showed that the increase in *Turicibacter* in chronic colitis might be a response to alleviate inflammation and that *Turicibacter* might have a protective effect on IBD, which requires further research.

Our research also found that selenium yeast can increase *Allobaculum*. It has been reported that *Allobaculum* is positively correlated with tight junction (TJ) protein and negatively correlated with DAI score and histology score.[47] The TJ protein plays an important role in the intestinal barrier.[48] Moreover, *Allobaculum* can metabolize tryptophan, produce aryl hydrocarbon receptor (AHR) ligands, activate AHR, produce IL-22, and alleviate intestinal inflammation.[49,50]

Our research also showed that selenium yeast can increase the abundance of *Bifidobacterium*. One study pointed out that *Bifidobacterium* strains inhibit inflammation by regulating the inflammatory pathway and could be used as a supplementary treatment for IBD.[51] The increase in *Bifidobacterium* was related to the success of anti-TNF-α treatment.[52] However, some studies have different opinions. The abundance of *Bifidobacterium* increased in UC patients, and the abundance in active UC was higher than that in UC in continuous remission.[53] It has also been reported that there was no significant difference in the abundance of *Bifidobacterium* between UC patients and healthy people.[54] Meanwhile, our research found that selenium yeast increased the abundance of *Prevotella*. It has been found that *Prevotella* is abundant in UC patients with continuous remission.[53] However, another study found that there is no significant difference in the abundance of *Prevotella* between UC patients and healthy people.[54] These opposite findings indicate that the gut microbiota might play a complex and dynamic role in the occurrence and development of IBD, which needs further research.

SCFAs are metabolites of gut microbiota, including acetate, propionate, butyrate, pentanoic (valuable) acid, and hexanoic (caproic) acid.[55] Butyric acid-producing bacteria mainly include *Lachnospiraceae, Ruminococcaceae* and some *Bacteroides*.[56] Butyric acid is the energy source of colon epithelial cells.[57] Our study found that butyric acid in the intestine of mice with DSS-induced chronic colitis decreased significantly ($P<0.05$). SCFAs in the intestine of IBD patients are different from those in normal people.[58] The metabolism of butyric acid in UC patients was abnormal, and butyric acid intake and oxidation decreased.[59] It has been reported that butyric acid decreases in active UC and increases in resting UC.[60] Butyrate can promote the production of IL-10,[61] inhibit the function of neutrophils, and alleviate the intestinal inflammation of mice with DSS-induced acute colitis.[62] IL-10 plays an important role in regulating intestinal homeostasis.[63] In addition, butyric acid could also regulate the repair of the intestinal mucus barrier.[64] Our study found that selenium yeast can significantly increase the content of butyric acid, which may be related to the effect of selenium yeast in alleviating chronic colitis.

Selenium yeast also has an effect on cytokines. Studies have reported changes in cytokines in IBD. TNF-α, IFN-γ, and IL-1β were elevated in patients with IBD.[65] Serum IL-17A was increased in active UC children when compared to those in remission.[66] We found that selenium yeast could decrease the TNF-α concentration. TNF-α plays an important role in the pathogenesis of IBD, and anti-TNF-α treatment reduces the production of proinflammatory factors by neutrophils.[67] Infliximab, an anti-TNF-α drug, has been widely used in the treatment of IBD. Neutralization of IL-6 and TNF-α decreased intestinal permeability and relieved DSS-induced acute colitis.[68] A clinical study found that an IL-6 antagonist had effects on patients with moderate to severe CD who experienced anti-TNF-α treatment failure.[69] IL-6 is an important proinflammatory factor.[70]

IL-1β plays an important role in the occurrence of intestinal inflammation. One study found that IL-1β can recruit immune cells (innate immune cells and CD4+ T cells) that secrete IL-17A.[71] IL-1β can also increase intestinal permeability and then promote the occurrence and development of intestinal inflammation.[72] Another study found that IL-1β upregulated MIR200c-3p, degraded occludin mRNA, and increased intestinal mucosal permeability.[73] In addition, IL-1β can activate mitogen-activated protein kinase kinase kinase MEKK-1, thereby activating the canonical NF-κB pathway and increasing the permeability of Caco-2 intestinal epithelial TJs.[74] IFN-γ can also affect the proliferation and apoptosis of intestinal epithelial cells and aggravate intestinal inflammation.[75] IFN-γ can induce chemokine C-X-C motif ligand 10 (CXCL10).[76] CXCL10 can promote the

development of colitis.[77] In addition, IFN-γ can also destroy the adherens junction protein vascular endothelial cadherin (VE-cadherin), damage the intestinal vascular barrier, and promote inflammation.[78]

Our study found that in mice with DSS-induced chronic colitis, IL-22 increased and selenium yeast significantly reduced IL-22. Different clinical studies have reported different changes in IL-22 in the serum of IBD patients. It has been reported that IL-22 in the serum of patients with active IBD was elevated.[79] However, another study found that serum IL-22 in IBD patients was reduced compared with the control group.[80] IL-22 has complex dual effects on intestinal homeostasis.[81] On the one hand, IL-22 can promote endoplasmic reticulum stress in intestinal epithelial cells, thereby aggravating intestinal inflammation.[82] In addition, IL-22 can promote intestinal fibrosis.[83] IL-22 is also related to the nonresponse to ustekinumab in UC patients.[84] On the other hand, IL-22 could enhance the integrity of the mucus barrier and promote the regeneration of intestinal epithelial cells.[85] Similar to IL-22, IL-17A has both protective and pathogenic effects in the intestine,[70] although our study found that selenium yeast can reduce the concentration of IL-17A. It has been reported that IL-17A plays an important role in DSS-induced acute colitis.[86] Moreover, IL-17A can enhance the role of IL-22 in promoting endoplasmic reticulum stress in intestinal epithelial cells, thereby aggravating inflammation.[82] IL-17A also has a protective effect on intestinal epithelial barrier function.[87] Meanwhile, a clinical study found that anti-IL-17A did not contribute to the remission of CD.[88] Therefore, the role of IL-17A and IL-22 in IBD needs further evaluation.

MPO is a kind of peroxidase containing hemoglobin, which is mainly expressed in neutrophils and less expressed in monocytes.[89] Our study found that MPO increased in the chronic colitis group. Compared with normal people, MPO in the stool of patients with IBD increased.[90] It has been found that fecal MPO can be used to assess the disease activity of UC and its response to treatment.[91]

Conclusion

In general, selenium yeast alleviated DSS-induced chronic colitis in mice by reducing proinflammatory factors and regulating the gut microbiota and metabolites. Cytokines, gut microbiota and their metabolites, such as SCFAs, play an important role in IBD. Drugs for these targets have been applied in treating IBD. In addition, selenium yeast is a common selenium supplement that is convenient to access. Therefore, selenium yeast might be a promising drug in the treatment of IBD.

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Disclosure

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