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Effects of inulin and isomalto-oligosaccharide on diphenoxylate-induced constipation, gastrointestinal motility-related hormones, short-chain fatty acids, and the intestinal flora in rats

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The prebiotics inulin (INU) and isomalto-oligosaccharide (IMO) influence intestinal health and immunity, but their effects on constipation are not clearly established. We evaluated the effects of INU and IMO in a rat model of diphenoxylate-induced constipation. Twenty-four male rats were divided into four groups: basal diet (Con), 40 mg kg^{-1} diphenoxylate (PCon), 20 g kg^{-1} INU and treated with 40 mg kg^{-1} diphenoxylate, and 20 g kg^{-1} IMO and treated with 40 mg kg^{-1} diphenoxylate. INU and IMO increased the number, weight, and water content of fecal pellets, and decreased the time to the first black stool in rats with constipation. Serum levels of the gastrointestinal motility-related hormones adrenocorticotropic hormone (ACTH), motilin (MTL), and Substance P (SP) were higher and corticosterone (CORT), vasoactive intestinal peptide (VIP), and calcitonin gene-related peptide (CGRP) were lower in rats treated with prebiotics than in untreated rats. Colon tissue levels of MTL and SP were increased, and VIP and CGRP were decreased by prebiotics. Furthermore, in rats with constipation, INU and IMO increased the colonic contents of short-chain fatty acids. The relative abundance of Bacteroidetes was lower in the prebiotics groups than in the Con and PCon groups. *Lactobacillus* was more abundant in the INU and IMO groups than in PCon rats. *Lactobacillus reuteri* and *Lactobacillus intestinalis* were more abundant in the IMO group than in the PCon group ($P < 0.01$), and *L. intestinalis* was more abundant in the INU group than in the PCon group ($P < 0.01$). In summary, INU and IMO improved constipation and altered the intestinal microbiota in a rat model of constipation.

Introduction

Constipation is a common gastrointestinal problem worldwide, affecting 5%–30% of the population, with increased incidences in females and older individuals.[1,2] Dietary fiber intake, mental state, and psychological morbidities can lead to constipation.[3] This condition is commonly characterized by infrequent stools, difficulty in passing stools, lumpy or hard stools, laborious defecation, incomplete evacuation and anorectal obstruction or blockage.[4,5] Constipation can lead to skin aging, headaches, pimples, hemorrhoids, and colorectal cancer.[6–8] Laxatives are commonly used for the treatment of constipation; however, their frequent use can have various adverse effects, such as severe dehydration and electrolyte disturbances.[9,10] Therefore, the identification of foods and natural products able to effectively prevent and treat constipation has emerged as a contemporary challenge.

Prebiotics are non-digestible substances, some of which (galacto-oligosaccharides and inulin-type fructans) affect the intestinal microbiota, intestinal peristalsis, or constipation.[11,12] Inulin (INU) is a non-digestible soluble dietary fiber derived mainly from the Jerusalem artichoke.[13] It consists of fructose subunits linked by 2,1 glycosidic linkages with a glucose terminal unit.[14] INU is a powerful prebiotic and a valuable alternative to antibiotic growth promoters.[15] A significant increase in stool frequency following INU ingestion has been reported in healthy individuals with constipation.[16] INU has a positive effect on growth performance, carcass yield, immune activity, and serum biochemical parameters in chickens.[14,17,18] Wang *et al.* reported that INU has beneficial effects on growth performance and carcass traits in growing-

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finishing pigs.[19] In addition, INU has positive effects on hyperglycemia in mice with high-fat-diet-induced diabetes[20] and in humans with type 1 and type 2 diabetes.[21]

Oligosaccharides contain 2–10 sugar units and usually have good water solubility and low viscosity.[22] Recently, enzyme-synthetic isomalto-oligosaccharide (IMO), one of the most common commercial oligosaccharides, has been identified as a promising new prebiotic in the global market.[23,24] According to Mizubuchi *et al.*, IMO activates the immune system of sows and protects sow health.[25] IMO influences metabolic alterations in high fat diet-induced mice by preventing gut dysbacteriosis.[26] In rats, IMO reduces the risk of colon cancer.[27] Moreover, Wu *et al.* have shown that IMO improves performance by strengthening immune function and intestinal health in weaned pigs.[28]

Despite several studies of the effects of INU and IMO on nutrition, immune health, and human diseases, the effects on animal constipation are unclear. Although inulin has been used as a prebiotic to relieve constipation to some extent, it mainly focuses on children, women and the elderly suffering from constipation, and no further research has been conducted on rigorous and accurate constipation models.[29–32] Therefore, in this study, the effects of INU and IMO on the defecation status and time, intestinal peristaltic hormone synthesis, SCFA production, and the intestinal microbial community in rats with diphenoxylate-induced constipation were evaluated.

Experimental

Animal treatment

Twenty-four male rats were purchased from the Zhejiang Academy of Medical Sciences (Hangzhou, China) and divided into four treatment groups (n = 6 per group). Rats were housed in a room with a constant temperature (25 ± 2 °C) and humidity (55 ± 10%). All rats consumed a standard diet and tap water for 1 week. After 1 week of adaptation, four groups of rats were treated as follows: standard diet (Con); 40 mg kg^{-1} diphenoxylate (PCon); 40 mg kg^{-1} diphenoxylate and 20 g kg^{-1} INU; 40 mg kg^{-1} diphenoxylate and 20 g kg^{-1} IMO. The experiment continued for 7 days. The dosage and treatment time of diphenoxylate in the experiment were obtained from our previous ADC-induced constipation model experiment in rats. All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Zhejiang A&F University and were approved by the Animal Ethics Committee of Zhejiang A&F University (SYXKzhe 2019-075).

Characteristics of fecal pellets and time to the first black stool

The number and weight of fecal pellets of every rat were determined at 8 a.m. during the experimental period. The fecal pellets were dried for 3 h at 90 °C and the water content was calculated according to the following formula: Fecal water content (%) = [(fecal wet weight − fecal dry weight)/fecal wet weight] × 100. Following 7 days of treatment, rats were fasted for 12 h and given 2 mL of 100 g L^{-1} activated carbon suspension intragastrically. The time to the first black stool was recorded for rats in every group.

Sample collection

After the first black stool was observed, rats were sacrificed after the collection of a blood sample from the heart. Serum samples were centrifuged immediately at 3000g for 10 min and stored at −80 °C. Each rat was dissected and colon tissue was collected, washed, and homogenized in a homogenizer with phosphate buffer (pH 7.4) to obtain a 1 : 9 (w/v) whole homogenate, followed by centrifugation at 3000g for 20 min at 4 °C. The supernatant was obtained and stored at −20 °C until further use. Colon contents were taken out on a sterile operation platform into a sterilized 10 ml centrifuge tube, and stored at −80 °C until use.

Biochemical analysis of serum and colon tissue samples

Reagent kits for adrenocorticotropic hormone (ACTH), corticosterone (CORT), calcitonin gene-related peptide (CGRP), motilin (MTL), Substance P (SP), and vasoactive intestinal peptide (VIP) were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). All kits were used in strict accordance with the operation manual.

Short-chain fatty acid (SCFA) analysis

SCFAs were measured as described in our previous report.[33] Briefly, colon contents were diluted with deionized water at a ratio of 1 : 9 (2 g of intestinal digesta was thawed and suspended in 2 mL of distilled water in a centrifuge tube), followed by repeated centrifuged at 10 000g at 4 °C for 10 min to obtain the supernatant. After vortexing and resting at room temperature for 30 min, the samples were centrifuged (5000g for 10 min at 4 °C). 1 mL of supernatant was transferred into 2 mL centrifuge tubes and mixed with 0.2 mL metaphosphoric acid (25%, w/v); it was left to stand at room temperature for 30 min, and the tubes were centrifuged again (10 000g for 10 min at 4 °C). One microliter of the supernatant was centrifuged at 10 000g for 4 min at 4 °C. Finally, the supernatants were filtered using a 0.22 μm membrane. SCFA concentrations in the aliquots of the supernatant (500 μL) were then analysed for gas chromatography. The Agilent 6890 N gas chromatograph (GC) system equipped with a flame ionization detector (FID) and a GC column (HP-INNOWAX, 190901N-213, Agilent Technologies, Santa Clara, CA, USA) of 30 m × 0.32 mm I.D. coated with a 50 mm-thick film was used. The carrier gas was nitrogen (flow rate, 19.0 mL min^{-1}). The injection temperature was 240 °C. The injected sample volume for the GC analysis was 200 μL, and the running time for each analysis was 20.5 min. The standard solutions (acetic, propionic, isobutyric, butyric, isovaleric, and valeric acids) were purchased by from Aladdin Reagent Co. Ltd (Shanghai, China).

HiSeq sequencing analysis of community structures

Metagenomic DNA from the colon content samples was obtained using the QIAamp DNA Stool Mini Kit (Qiagen

GmbH, Hilden, Germany) following the manufacturer's instructions. The concentration and purity of the extracted DNA were measured using the Nanodrop 1000 UV-Vis Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA), and the quality and purity of the extracted DNA were confirmed by 1% agarose gel electrophoresis. The V4 region of 16S rRNA was PCR-amplified using a forward primer (5′-ACTCCTACGGGAGGCAGCA-3′) and reverse primer (5′-GGACTACHVGGGT WTCTAAT-3′). The desired PCR products from each DNA sample were purified using the Qiagen QIAquick Gel Extraction Kit (Qiagen, Carlsbad, CA, USA) after agarose gel electrophoresis. All libraries were subsequently pooled with equal molar concentrations and sequenced using the Illumina HiSeq system (Novogene Bioinformatics Technology Co., Ltd, Beijing, China).

Statistical analysis

Parameter values were compared among groups using one-way analysis of variance (ANOVA) with SPSS 25 (SPSS Inc., Chicago, IL, USA). A value of $P < 0.05$ was considered statistically significant. Histograms were generated using GraphPad Prism 6 (GraphPad Prism Inc., La Jolla, CA, USA).

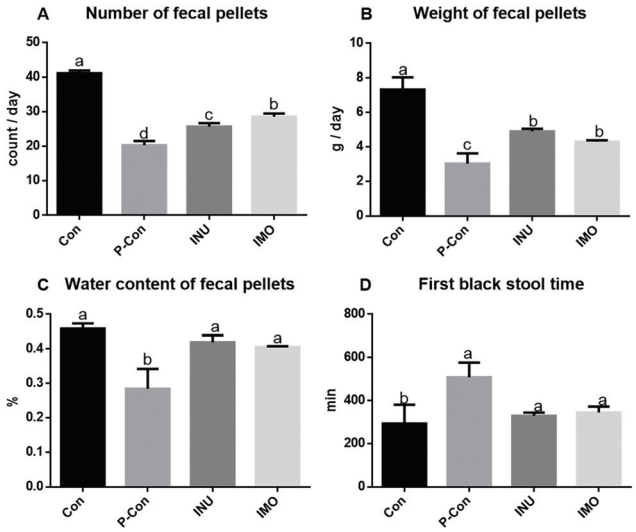

Fig. 1 Effect of inulin and isomalto-oligosaccharide on the fecal parameters in rats: (A–D) the number, weight and water content of rat fecal pellets, and first black stool time, respectively. Con represents control rats; P-Con represents rats with diphenoxylate-induced constipation; INU represents rats with diphenoxylate-induced constipation supplemented with inulin; IMO represents rats with diphenoxylate-induced constipation supplemented with isomalto-oligosaccharide. Different letters indicate a significant difference ($P < 0.05$). Means values ($n = 6$) were used for the analysis of fecal parameters.

Results

Fecal parameters

The rats treated with diphenoxylate had significant reductions ($P < 0.05$) in the number, weight and water content of fecal pellets compared with those of untreated rats as well as a significantly extended time to the first black stool ($P < 0.05$). Diphenoxylate-treated rats treated with INU or IMO had significant increases ($P < 0.05$) in the number, weight, and water content of fecal pellets compared with those in the PCon group (Fig. 1A–C). The water contents of fecal pellets in the INU and IMO groups were not significantly different ($P > 0.05$) from that of the Con group. The time to the first black stool was significantly shorter in the INU or IMO group than in the PCon group ($P < 0.05$) (Fig. 1D). Analyses of fecal parameters indicated that INU and IMO can relieve constipation in rats.

Biochemical analysis of serum samples

As shown in Fig. 2, we found that the serum levels of ACTH, VIP, and CGRP were significantly higher ($P < 0.05$) and the serum levels of CORT, MTL and SP were significantly lower ($P < 0.05$) in diphenoxylate-treated rats than in control rats. This indicated that diphenoxylate-induced constipation could affect the secretion of related hormones. In addition, INU and IMO significantly reduced ($P < 0.05$) the serum ACTH, VIP, and CGRP levels, and increased ($P < 0.05$) the serum CORT, MTL, and SP levels compared with those of the rats in the PCon group. INU and IMO have positive effects on hormonal disorders related to constipation in rats.

Biochemical analysis of colon tissue samples

Compared with the Con group, rats administered diphenoxylate had distinctly lower levels ($P < 0.05$) of MTL and SP secretion, and higher ($P < 0.05$) VIP and CGRP in colon tissues. Furthermore, the rats in the INU or IMO group exhibited substantial increases ($P < 0.05$) in MTL and SP levels, and significantly decreased ($P < 0.05$) VIP and CGRP levels in colon tissues (Fig. 3). Additionally, the levels of VIP in the colon tissues of rats in the INU and IMO groups were similar to those in the Con group ($P > 0.05$) (Fig. 3C). INU and IMO positively regulated rat colon tissue-related hormones, consistent with the results of serum analyses.

Concentrations of SCFAs

As shown in Fig. 4, diphenoxylate-induced constipation significantly reduced SCFAs ($P < 0.05$) in rat colons. However, the concentrations of butyric, propionic, butyric, isobutyric, and valeric acids in the colonic contents of rats treated with INU or IMO were significantly higher ($P < 0.05$) than those in the PCon group, while the concentration of isovaleric acid did not differ significantly ($P > 0.05$) between the PCon group and IMO or INU groups (Fig. 4). These results indicate that INU and IMO promote the secretion of SCFAs in the intestine.

Microflora structure in the colon contents

In this study, 24 colonic content samples from the four treatment groups were studied to evaluate the mechanisms underlying the attenuation of diphenoxylate-induced constipation by INU and IMO. High-throughput sequencing using the Illumina

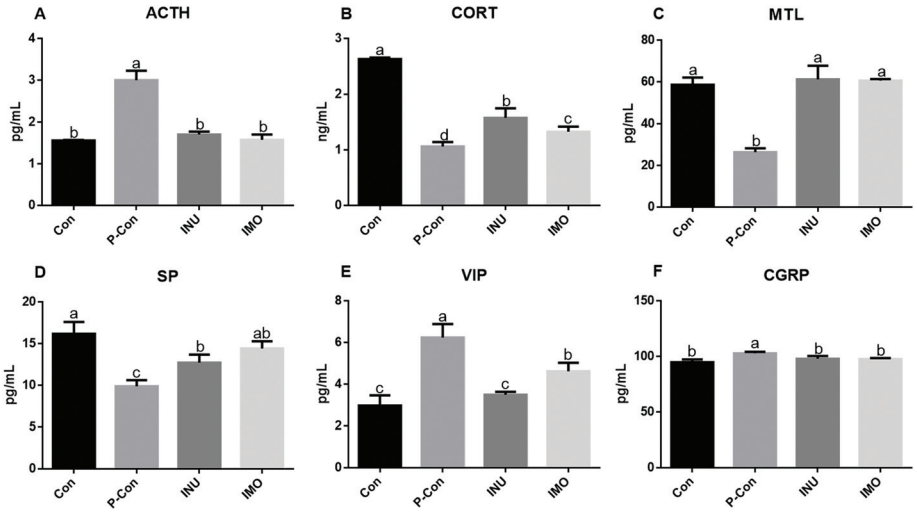

Fig. 2 Effects of inulin and isomalto-oligosaccharide on the serum gastrointestinal motility-related hormones in rats: (A–F) the content of ACTH, CORT, MTL, SP, VIP, and CGRP in rat serum, respectively. Con represents control rats; P-Con represents rats with diphenoxylate-induced constipation; INU represents rats with diphenoxylate-induced constipation supplemented with inulin; IMO represents rats with diphenoxylate-induced constipation supplemented with isomaltooligosaccharide. Different letters indicate a means significant difference ($P < 0.05$). Mean values ($n = 6$) were used for the analysis of serum gastrointestinal motility-related hormones.

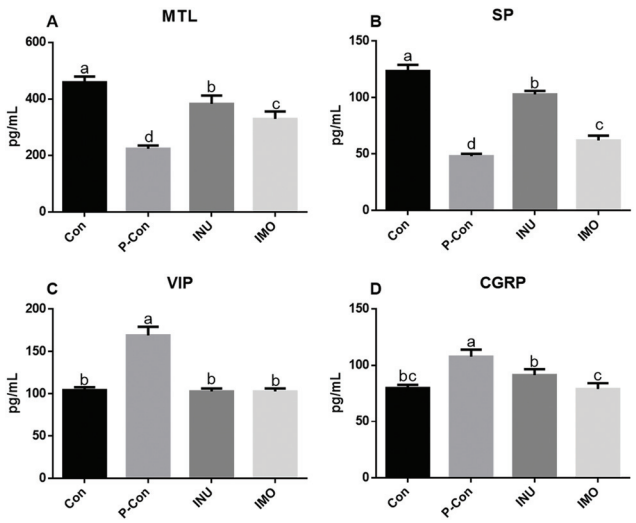

Fig. 3 Effect of inulin and isomalto-oligosaccharide on gastrointestinal motility-related hormones in the colon tissue of rats: (A–D) the content of MTL, SP, VIP, and CGRP in rat colon tissue, respectively. Con represents control rats; P-Con represents rats with diphenoxylate-induced constipation; INU represents rats with diphenoxylate-induced constipation supplemented with inulin; IMO represents rats with diphenoxylate-induced constipation supplemented with isomaltooligosaccharide. Different letters indicate significant differences ($P < 0.05$). Mean values ($n = 6$) were used for the analysis of colon tissue gastrointestinal motility-related hormones.

HiSeq platform was performed based on 16S rDNA. A total of 865 operational taxonomic units (OTUs) were shared among the four treatment groups, while the Con group had 21 unique OTUs, the PCon group had 10 unique OTUs, the INU group had 48 unique OTUs, and the IMO group had 32 unique OTUs (Fig. 5A). However, levels of diversity based on the Shannon index and β-OTU in the INU or IMO group's rats were lower than those in the Con and PCon groups (Fig. 5B and C). Moreover, in an analysis of the microbial composition of colonic contents in rats, Firmicutes and Bacteroidetes were the dominant bacterial phyla, and the abundance of Bacteroidetes was higher in the prebiotics groups than in the Con and PCon groups. (Fig. 5D). At the family level, we observed that Lactobacillaceae, Lachnospiraceae, Peptostreptococcaceae, Ruminococcaceae, and Muribaculaceae were the dominant families, and Lactobacillaceae was more abundant in the PCon group rats than in other groups (Fig. 5G). At the genus level, *Lactobacillus*, *Romboutsia*, *Lachnospiraceae*, *Turicibacter*, and *Ruminococcaceae* were dominant in all samples (Fig. 5H). At the species level, the abundances of *Lactobacillus reuteri* and *Lactobacillus intestinalis* were higher in the IMO group than in the PCon group ($P < 0.01$), and the abundance of *Lactobacillus intestinalis* was higher in the INU group than in the PCon group ($P < 0.01$) (Fig. 5E and F).

Discussion

Many recent studies have focused on constipation. Wang *et al.* found that dietary supplementation with 0.75 g kg^{-1} L-arabinose promotes intestinal motility, shortens the time to first defecation, and increases fecal weight and the number of fecal grains in mice.[34] In rats with loperamide-induced constipation, the number, wet weight, and water content of fecal pellets in taurine-xylose-supplemented rats are markedly higher than those in control rats.[35] *Houttuynia cordata Thunb.* extract increases the water content of fecal pellets but has no effect on the number of fecal pellets in rats with constipation.[36] The water content of feces increases with the intake of oligosaccharides.[37] Dual-Oligo increases the fecal weight

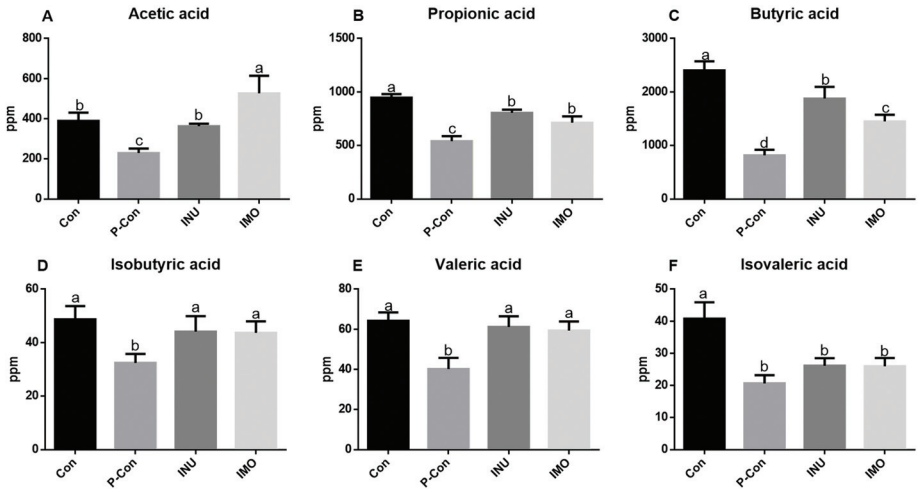

Fig. 4 Effect of inulin and isomalto-oligosaccharide on the SCFAs in the colon content of rats: (A–F) the content of acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, and isovaleric acid in rat colon content, respectively. Con represents control rats; P-Con represents rats with diphenoxylate-induced constipation; INU represents rats with diphenoxylate-induced constipation supplemented with inulin; IMO represents rats with diphenoxylate-induced constipation supplemented with isomalto-oligosaccharide. Different letters indicate a significant difference ($P < 0.05$). Mean values ($n = 6$) were used for the analysis of SCFAs.

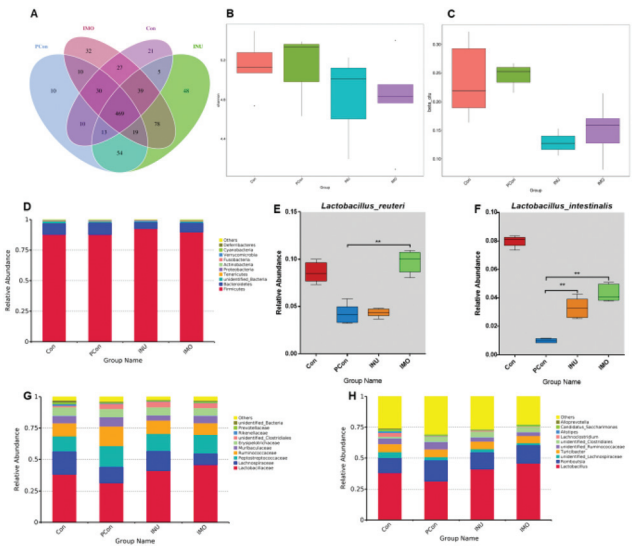

Fig. 5 Summary of the microbial community composition in the colon contents of rats. (A) Venn diagram summarizing the numbers of common and unique operational taxonomic units (OTUs) in the microflora community in the colonic contents of rats. (B) Shannon index, reflecting species diversity within and between groups. (C) β-OTU index, reflecting species diversity within and between groups. (D, G, H) Top 10 taxa with differences in relative abundance between groups (phylum, family, and genus levels). (E and F) Species with significant differences between groups. ** $P < 0.01$.

and water content of feces and the gastrointestinal transit ratio in rats with loperamide-induced constipation.[38] Our results further showed that INU and IMO increased the number, weight, and water content of fecal pellets, shortened the time to the first black stool in rats with constipation induced by diphenoxylate, and significantly alleviated constipation.

MTL, SP, VIP, ACTH, CORT, and CGRP are important indicators of intestinal peristalsis. Zhang *et al.* have shown that electroacupuncture inhibits CGRP and SP in the colons of rats with constipation-predominant irritable bowel syndrome.[39] In rats with functional constipation, manual acupuncture and electroacupuncture decrease the colon tissue expression of CGRP, TRPV 1, and PAR-4 at the protein and gene levels.[40] Healthy males administered Bushi-richu-to (a traditional Japanese Kampo medicine) exhibit significant increases in plasma CGRP, SP, VIP, and somatostatin-IS levels compared with those in a placebo control group.[41] Jia *et al.* have shown that loperamide hydrochloride-induced increases in VIP levels in rat plasma and colonic tissues are attenuated by filiform needling, electroacupuncture, and moxibustion treatment.[42] Additionally, 10% and 15% high specific volume polysaccharide reduces serum VIP levels and effectively alleviates diphenoxylate-induced constipation in rats.[43] Total glucosides of paeony attenuate atropine-diphenoxylate-induced slow transit constipation responses, as evidenced by reductions in NO, NOS, and VIP in the serum.[44] In addition, in rats with loperamide-induced constipation supplemented with taurine-xylose, the serum levels of gastrin, motilin, and somatostatin improve substantially.[35] Additionally, the serum levels of MTL, gastrin (Gas), and SP are significantly higher in rats with *Cordyceps militaris* polysaccharide-supplemented constipation than those in untreated rats.[45] Furthermore, Li *et al.* have shown that *Anemarrhena asphodeloides* Bge. polysaccharides significantly increase the levels of Gas, MTL, SP, and 5-hydroxytryptamine (5-HT) and decrease the NO content in loperamide-induced rats to ameliorate constipation.[46] In rats administered with rhubarb, the serum levels of MTL and Gas were attenuated to varying degrees, while the variation in VIP was not consistent.[47] In a similar study by Liu *et al.*, mice with diphenoxylate-induced

constipation fed with different doses of Konjac mannan oligosaccharides exhibited increased serum MTL and SP, decreased acetylcholinesterase (AchE), and no differences in VIP compared with those in mice with diphenoxylate-induced constipation.[48] Furthermore, *Lactobacillus fermentum* improves the levels of MTL, Gas, AchE, SP, and VIP in the serum of mice with activated-carbon-induced constipation.[49] In this study, INU and IMO increased the serum and colon tissue levels of MTL and SP, decreased VIP and CGRP, increased the serum levels of CORT and decreased serum ACTH in rats with diphenoxylate-induced constipation. Corticosterone is a negative regulator of ACTH secretion; the observed increase in ACTH likely reflects the feedback inhibition of the hypothalamic-pituitary-adrenocortical axis by corticosterone.[50]

Constipation causes a significant reduction in SCFA production and an increase in iso-butyrate.[51] Konjac mannan oligosaccharides increase the concentration of SCFAs and lactic acid in the feces of mice with diphenoxylate-induced constipation.[48] Hydrolyzed guar gum promotes the production of SCFAs in mice with constipation.[52] Constipated rats fed with Durio zibethinus Murr rind polysaccharide can increase the content of SCFA in the intestines and improve the gastrointestinal peristalsis.[53] *Lactobacillus plantarum* supplementation increases the SCFA levels (acetate, propionate, and butyrate) in women with functional constipation.[54] SCFA in *Lactobacillus plantarum* NCU116-treated mice with constipation are obviously higher than those in mice with constipation.[55] In addition, *Lactobacillus paracasei* CNCM I-1572 induces a significant increase in SCFAs, mainly acetate and butyrate.[56] INU is readily fermentable by intestinal bacteria, generating large quantities of SCFAs.[57] SCFAs stimulate ileal propulsive contractions by evoking prolonged propagated contractions and discrete clustered contractions. The possible mechanisms by which SCFAs mediate gut motility may involve the intestinal release of 5-HT. Furthermore, SCFAs could directly stimulate ileal and colonic smooth muscle contractility.[58] In the present study, diphenoxylate-induced constipation reduced colonic SCFAs. INU and IMO promoted the secretion of SCFAs in the colons of rats with diphenoxylate-induced constipation.

Constipation affects individuals of all ages and its occurrence is tightly linked with alterations in the gut microbiota.[59] More importantly, there is a bidirectional relationship between the gut microbiota and constipation.[60] In humans, chronic constipation can be characterized by a relative decrease in obligate bacteria (*e.g.*, *Lactobacillus*, *Bifidobacterium*, and *Bacteroides* spp.) and a parallel increase in potentially pathogenic microorganisms (*e.g.*, *Pseudomonas aeruginosa* and *Campylobacter jejuni*).[61,62] Khalif *et al.* reported that the levels of *Bifidobacterium* and *Lactobacillus* are significantly decreased in adult patients with constipation.[63] Our results showed that the abundance of *Lactobacillus* is lower in rats with constipation than in healthy rats, consistent with the results of Khalif *et al.* Galacto-oligosaccharide (GOS) and xylo-oligosaccharide (XOS) predominantly promote the accumulation of Bacteroidetes and inhibit the growth of *Desulfovibrio* in the feces with constipation.[52] Studies of the transfer of conventional intestinal microbiota to germ-free rats have revealed that *Lactobacillus acidophilus* and *Bifidobacterium bifidum* could accelerate small intestinal transit, while *Micrococcus luteus* and *Escherichia coli* show an inhibitory effect.[64] Shen *et al.* found that *Lactobacillus* increases the relative abundance of beneficial bacteria in the mouse fecal flora and improves the structure of the fecal flora as well as SCFA levels.[65] Zengye decoction, a traditional Chinese medicinal formula consisting of Radix scrophulariae, Ophiopogon japonicus, and Radix rehmannia, reduces the levels of harmful bacteria, such as *Desulfovibrio*, *Ruminococcus*, *Prevotella* and *Dorea*, and increases the abundance of *Oxalobacter*, *Clostridium*, and *Roseburia* in aged constipated rats.[66] Dietary fiber from sweet potato residue used to feed Wistar rats mediated a significant increase in the concentrations of *Bifidobacterium* and *Lactobacillus*, whereas it induced a significant decrease of *Enterobacillus*, *Clostridium perfringens* and *Bacteroides*.[67] Moreover, the result obtained by Chen *et al.* showed that one water-soluble polysaccharide from Ginkgo biloba leaves reversed gut dysbiosis, increased the richness of *Lactobacillus* species, and maintained the balance of intestinal flora.[68] Lycium barbarum polysaccharide increased the abundance of the phyla Proteobacteria and Firmicutes, while it reduced the ratio of the phylum Bacteroidetes and stimulated the emergence of some potential probiotic genera (*Akkermansia*, *Lactobacillus*, and *Prevotellaceae*).[69] In addition, rats administrated with galacto-oligosaccharide induced a significant change in the beta diversity of the gut microbiome and proliferation of *Bifidobacterium* and other potentially anti-inflammatory microbes.[70] Jiang *et al.* indicated that Durio zibethinus Murr rind polysaccharide increased the abundance of *Lachnospiraceae-NK4A136* in the intestines of loperamide hydrochloride-induced constipated rats, while it reduced the abundance of *Desulfovibrio*.[53] Therefore, we hypothesized that the reduction of harmful intestinal bacteria led to a reduction in bacterial diversity, as supported by our analyses of Shannon and β-OTU diversity indexes. In our study, although INU and IMO reduced the diversity of the gut microbiota, they significantly increased the levels of beneficial bacteria in the flora, especially *Lactobacillus*. IMO significantly increased *L. reuteri* and *L. intestinalis*, and INU significantly increased *L. reuteri*, a Gram-positive bacterium that can be found in the digestive tract of many vertebrates, including humans, pigs, rodents, sheep, cattle, and birds.[71] Duar *et al.* and Oh *et al.* revealed that some *L. reuteri* has a competitive advantage over other bacteria in the rodent gastrointestinal tract.[72,73] Moreover, *L. reuteri* DSM 17938 maintains the balance in the gastrointestinal microbiome, and is effective in the management of functional constipation in young children.[74]

These findings indirectly demonstrated that the intestinal microbiota could regulate endocrine cells and ultimately influence gut motility. Bifidobacteria and lactobacilli are generally recognized as beneficial taxa with various health-promoting functions, such as the promotion of the production of SCFAs able to stimulate motilin, the stimulation of intestinal peristalsis, and the ability to increase the water content of the fecal bolus.[75]

Collectively, the altered intestinal microbiota can regulate gastrointestinal motility and alleviate constipation by affecting the secretion of gastrointestinal hormones and SCFA levels.

Conclusions

In conclusion, our study proved that INU and IMO increase the number, weight, and water content of fecal pellets, decrease the time to the first black stool, increase the serum and colon tissue levels of MOT and SP, decrease VIP and CGRP, increase the serum levels of ACTH, decrease serum CORT, improve the levels of SCFAs in the colon, increase colonic *Lactobacillus* abundance, and optimize microbial stability. In addition, INU and IMO may regulate gastrointestinal motility-related hormones and SCFA secretion by optimizing colonic microbial abundances in rats with diphenoxylate-induced constipation, thereby alleviating constipation.

Conflicts of interest

This work has no conflict of interest.

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