Clinical characteristic and fecal microbiota responses to probiotic or antidepressant in patients with diarrhea-predominant irritable bowel syndrome with depression comorbidity: a pilot study

Lu Zhang1, Yi-Xuan Liu1, Zhe Wang2, Xiao-Qi Wang2, Jing-Jing Zhang1, Rong-Huan Jiang3,4, Xiang-Qun Wang3, Shi-Wei Zhu1, Kun Wang1, Zuo-Jing Liu1, Huai-Qiu Zhu2, Li-Ping Duan1

1Department of Gastroenterology, Peking University Third Hospital, Beijing 100191, China; 2Department of Biomedical Engineering, College of Engineering, and Center for Quantitative Biology, Peking University, Beijing 100871, China; 3Department of Psychiatry, Institute of Mental Health, Peking University, Beijing 100191, China; 4Department of Psychological Medicine, Chinese People's Liberation Army (PLA) General Hospital, Beijing 100853, China.

To the Editor: Irritable bowel syndrome (IBS) is a prevalent functional gastrointestinal disorder that presents as abdominal pain with altered bowel habits. The pathophysiologic mechanism of IBS is not well understood, although many hypotheses have been proposed, including visceral hypersensitivity, gastrointestinal dysmotility, low-grade inflammation of the intestinal mucosa, and dysfunction of the brain-gut interaction. Dysfunction of the brain-gut interaction is thought to be involved in IBS because a considerable proportion of patients with IBS have some form of psychologic comorbidity, such as depression or anxiety. In addition, mental and psychologic problems increase the risk for IBS and the symptom severity of IBS. Recently, changes in the gut microbiota have also been suggested to contribute to both IBS and depression. Our previous study demonstrated significant altered gut microbiota profiles in patients with diarrhea-predominant IBS (IBS-D) and depression.1

Therapies that intend to manipulate the gut microbiota can frequently alleviate IBS symptoms. Probiotics are the most frequently used of these kinds of agents, and can shift the composition of the gut microbiota while relieving IBS symptoms. Shifts in the composition of gut microbiota and short-chain fatty acids (SCFAs) levels (the main metabolic products of gut bacteria) can result in the regulation of host immune function, which is essential in the pathogenesis of IBS. Antidepressants are often employed when patients with IBS have psychologic comorbidities, and are highly effective for the alleviation of both psychologic and IBS symptoms. However, whether probiotics or antibiotics can relieve the severity of psychologic comorbidities in IBS, in addition to the effect of antidepressants on the gut microbiota, are still unknown. Therefore, to investigate the interaction between the gut microbiota and the brain-gut axis, Duloxetine or probiotics were applied on patients with IBS-D with depression to identify the shifts in gut microbiota profiles and fecal SCFA levels, systematic inflammatory responses, and clinical responses of those patients to both therapies to clarify the potential mechanisms of the microbiota-gut-brain axis.

The protocol was approved by Peking University Third Hospital Medical Ethics Committee (No. 2013-112) and all the methods were carried out in accordance with the approved guidelines of the committee and with the Declaration of Helsinki. All patients provided written informed consent to participate. The present study has been registered on Chinese Clinical Trial Registry (No. ChiCTR-COC-17011176).

Patients with IBS-D (diagnosed according to the Rome III criteria) and depression (according to the Mini-International Neuropsychiatric Interview [MINI] DSM-IV version 5.0.0) comorbidity between 18 and 65 years were recruited from Department of Gastroenterology, Peking University Third Hospital and the Outpatient Department, Institute of Mental Health of Peking University. After endocrine, metabolic, infectious diseases and organic intestinal diseases were excluded, eligible participants were randomized (random number table) to receive probiotics (Biﬁdo®, SINE, Shanghai, China), 20mg 3 times daily, Biﬁco contains 3 strains: Bifidobacterium longum, Lactobacillus acidophilus, Enterococcus faecalis, and the number of bacteria is no less than 1.0×10^7 colony-forming units (CFUs) or antidepressants (Duloxetine [Cymbalta®, Eli Lilly and Company, USA] 30mg once daily for the first

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Correspondence to: Prof. Li-Ping Duan, Department of Gastroenterology, Peking University Third Hospital, Beijing 100191, China E-Mail: Duanlp@bjmu.edu.cn

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4 days, and raise to 60mg once daily if it was well tolerated) for 8 weeks. Data on the severity of IBS symptoms and depression, in addition to fecal and blood samples were collected before colonoscopy and at the end of treatment. The severity of IBS symptoms and depression was assessed using the IBS-severity scoring system (IBS-SSS) and the validated Zung self-rating depression scale (SDS), respectively and plasma cytokines, fecal SCFAs, gut microbiota composition were assessed before and after treatment.

Fecal samples were collected before and at the end of treatment and stored at −80°C immediately until DNA isolation was performed. Fecal bacterial DNA was isolated with the MoBio Power Soil DNA isolation kit (MO BIO Laboratories, Inc., CA, USA). The V1-V3 regions of the bacterial 16S ribosomal RNA gene were amplified using universal primers 27F and 533R and sequenced using the Roche 454 GS FLX+ Titanium platform (Roche 454 Life Sciences, Branford, CT) according to standard protocols.

To assess systematic inflammation, 11 plasma cytokines, including tumor necrosis factor-α, interleukin-10 (IL-10), interleukin-12 (IL-12), interleukin-6 (IL-6), interferon-γ (IFN-γ), interleukin-8 (IL-8), monocyte chemotactic protein-1 (MCP-1), monocyte chemotactic protein-3 (MCP-3), interleukin-23 (IL-23), interleukin-1β (IL-1β), and macrophage inflammatory protein-1α (MIP-1α), were measured using a Human Magnetic Luminex Screening Assay (11 PLEX; R&D LSXAHM-11).

Seven fecal SCFAs, including formate, acetate, propionate, butyrate, isobutyrate, valerate, and isovalerate, were assessed using an isotope-labeled chemical derivatization method on ultra-performance liquid chromatography-tandem mass spectrometry with a slightly modified method.[2]

All reference bacterial 16S rRNA sequences from RDP resources were downloaded and assembled into a database. The blastall function in BLAST was used to perform slow and accurate alignment with an e-value ≤ 10−5. The assignment number at the level of the genus of each sample was calculated.

Comparisons of age, body mass index (BMI), and severity of IBS symptoms and depression at baseline were conducted using Mann-Whitney U-test, while comparison of the gender ratio between the two groups was conducted using Fisher's exact test. Comparisons of the relative abundances of individual bacterial species, symptom severity scores, and fecal SCFA levels before and after treatment were conducted using the Wilcoxon signed rank test. For Luminex assay data analysis, duplicate readings were averaged for each standard and sample, and the average blank median fluorescence intensity (MFI) was subtracted. The concentration of each cytokine was calculated by creating a standard curve using the MFI of the pre-treatment values [Figure 1I].

The blastall function in BLAST was used to perform partial least square-discriminant analysis with the “mixOmics” package in R. All statistical tests were conducted in a two-tailed manner, and a P-value <0.05 was considered statistically significant. Throughout this study, data are presented as mean ± standard errors (SE) unless stated otherwise.

A total of 162 participants were screened and 15 patients with IBS-D with depression comorbidity were recruited to this study. Nine of them received Bifidobacterium and the other 6 received Duloxetine. Age, BMI, and the severity of IBS symptoms and depression were similar between the 2 groups. One patient in the Bifidobacterium group was excluded for taking antibiotics due to acute prostatitis. Fourteen patients completed the 8-week therapy period.

Of the patients treated with Bifidobacterium, both the total score of IBS-SSS (170.8 ± 23.0 vs. 281.6 ± 22.3, Z = −2.39, P = 0.017) and the component scores of IBS-SSS, including abdominal pain (28.9 ± 6.2 vs. 42.1 ± 7.5, Z = −2.02, P = 0.043), bloating (19.3 ± 6.4 vs. 58.6 ± 9.3, Z = −2.39, P = 0.017), satisfaction with bowel habits (51.4 ± 9.3 vs. 71.4 ± 7.2, Z = −2.37, P = 0.018), and interference with quality of life (33.8 ± 8.1 vs. 68.3 ± 9.7, Z = −2.53, P = 0.012) decreased significantly when compared with the pre-treatment values [Figure 2C and 2D]. When we classified IBS severity into 3 different levels according to the total score of IBS-SSS as recommended by the creator, the severity of 3 patients improved from severe to moderate, one from severe to mild, and 2 from moderate to mild, while 2 experienced no change. The maximum number of bowel movements per day did not change compared with that at pre-treatment [Figure 1C]. Meanwhile, the SDS score showed a decreasing trend (51.9 ± 4.9 vs. 64.0 ± 4.0, Z = −1.96, P = 0.05) [Figure 1D].

Of the patients treated with Duloxetine, a decrease in SDS score was noted (39.3 ± 3.5 vs. 64.0 ± 6.8, Z = −2.02, P = 0.043), and the total score of IBS-SSS (98.8 ± 52.4 vs. 282.3 ± 43.1, Z = −2.20, P = 0.028) as well as the component scores, including abdominal pain (4.2 ± 3.3 vs. 26.0 ± 6.0, Z = −2.03, P = 0.042), onset frequency of pain (18.3 ± 16.4 vs. 63.3 ± 14.8, Z = −2.03, P = 0.042), satisfaction with bowel habits (38.3 ± 15.1 vs. 76.7 ± 12.3, Z = −2.03, P = 0.042), and interference with quality of life (28.3 ± 16.4 vs. 78.3 ± 10.8, Z = −2.21, P = 0.027) decreased significantly compared with the pre-treatment values [Figure 1G, 1H, and 1J]. When classified into different severity levels according to the total IBS-SSS score, 1 patient improved from severe to mild, I from severe, 2 from moderate, and 1 from mild to complete remission. One patient experienced no change. The maximum number of bowel movements per day did not change compared with the pre-treatment values [Figure 1I].

A total of 279,518 reads (range 2264–205,099,983 reads per sample on average) and 23,407 operational taxonomic units (OTUs) (range 242–2,481,836 OTUs per sample on average) were obtained. The α diversity of gut microbiota did not change after treatment in any group [Figure 2A and 2B]. The overall gut microbiota structure was altered in the probiotic and Duloxetine treatment groups [Figure 2C and 2D].
Figure 1: Symptoms and plasma cytokines alteration at pre-treatment and post-treatment of patients with IBS-D with depression treated with Bifico (n=8) (A–F) or duloxetine (n=6) (G–L). IBS-D: Diarrhea-predominant IBS; IBS-SSS: Irritable bowel syndrome-severity scoring system; SDS: Zung self-rating depression scale.

Figure 2: Gut microbiota alteration of α diversity and structure in pre-treatment and post-treatment of patients with IBS-D with depression treated with Bifico (n=8) (A and C) or duloxetine (n=6) (B and D). IBS-D: Diarrhea-predominant irritable bowel syndrome.
In terms of variations in the relative abundances of bacteria, the relative abundance of Dialister increased and that of Clostridium XVIII and Bifidobacterium decreased significantly after treatment with Bifico, while that of Acetivibrio, Collinsella, and Odoribacter showed an increasing trend. Interestingly, the relative abundance of Fecalibacterium, Lachnospiraceae incertae sedis, Escherichia/Shigella, and Sutterella tended to increase and Erysipelotrichaceae incertae sedis tended to decrease in the Duloxetine group.

The plasma levels of MCP-1 decreased significantly in both the Bifico (86.76 ± 13.25 vs. 125.40 ± 18.48, Z = −1.99, P = 0.046) and Duloxetine post-treatment groups (53.71 ± 5.82 vs. 77.24 ± 8.95, Z = −2.20, P = 0.028) compared to pre-treatment values [Figure 1E and 1K]. The plasma level of IL-1β presented a significant decrease in the Bifico group (3.22 ± 0.06 vs. 10.53 ± 7.02, Z = −2.20, P = 0.028) and a trend to decrease in the Duloxetine group [Figure 1F and 1H]. Other plasma cytokine levels did not change significantly with treatment.

The overall composition of the 7 SCFAs in stool changed slightly in both groups. However, there were no significant changes in any single SCFA and the total levels of these seven fecal SCFAs in both groups, although fecal formate level tended to increase in the Duloxetine group [Supplementary Table 1, http://links.lww.com/CM9/A10].

Correlations between the relative abundances of some bacteria at genus level and the severity of symptoms, plasma cytokine levels, and fecal SCFA levels were observed.

Fecal SCFA levels were correlated with numerous gut bacteria, especially in the case of isobutyrate, valerate, and isovalerate. The majority of these correlations were positive, except for that between formate and Lachnospiraceae incertae sedis (r = −0.434, P = 0.022) or acetate and Flavonifractor (r = −0.386, P = 0.042).

Significant correlations between plasma cytokine levels and gut bacteria were also observed. Most of these relationships were positive, except for that between plasma IFN-γ and Roseburia (r = 0.581, P = 0.004), IL-1β and Clostridium XIVb (r = 0.410, P = 0.047), Catenibacterium (r = 0.492, P = 0.015), and Bifidobacterium (r = 0.408, P = 0.048); and MIP-1α and Parasutterella (r = 0.852, P = 0.015), Catenibacterium (r = 0.802, P = 0.030), and Gemmiger (r = 0.775, P = 0.041). MCP-1, was negatively correlated with Barnesiella (r = −0.510, P = 0.011), Odoribacter (r = −0.467, P = 0.021), and Sutterella (r = −0.574, P = 0.003).

Significant correlations were also observed between fecal SCFAs and plasma cytokines. Most of these correlations were positive. Fecal acetate was positively correlated with IL-6 (r = 0.437, P = 0.033), while fecal propionate (r = 0.857, P = 0.024) and the total level of all 7 fecal SCFAs (r = 0.821, P = 0.034) correlated positively with MIP-1α; fecal butyrate was positively correlated with IL-12 (r = 0.471, P = 0.020), MCP-3 (r = 0.605, P = 0.037), and MIP-1α (r = 0.786, P = 0.048); while fecal isobutyrate was negatively correlated with IFN-γ (r = −0.415, P = 0.049). There was also a positive correlation between fecal formate and IL-10 (r = 0.409, P = 0.047), which is an anti-inflammatory cytokine.

As peripheral inflammation might play an essential role in both IBS and depression, correlations between plasma cytokines and symptoms were analyzed further. The plasma MCP-1 level was positively correlated with abdominal pain (r = 0.448, P = 0.028), and IL-23 was positively correlated with the satisfaction with bowel habits (r = 0.532, P = 0.007).

Correlations between fecal SCFAs and symptoms were also analyzed. Fecal acetate was negatively correlated with abdominal bloating (r = −0.381, P = 0.046), interference with quality of life (r = −0.395, P = 0.038), and total IBS-SSS score (r = −0.392, P = 0.039), and fecal propionate was negatively correlated with abdominal bloating (r = −0.380, P = 0.046). Fecal valerate was positively correlated with satisfaction with bowel habits (r = 0.394, P = 0.038).

To obtain a more comprehensive view of the correlations between the relative abundances of gut bacteria, fecal SCFAs, plasma cytokines, and symptoms, correlation network analysis was conducted and three interesting networks were found. In the 1st network [Supplementary Figure 1A, http://links.lww.com/CM9/A10], plasma MCP-1 was positively correlated with abdominal pain severity but negatively correlated with the relative abundances of Barnesiella, Odoribacter, and Sutterella. Additionally, depression severity (SDS scores) was positively correlated with abdominal pain severity. In the second network [Supplementary Figure 1B, http://links.lww.com/CM9/A10], Flavonifractor was negatively correlated with fecal acetate, and the latter was negatively correlated with bloating and IBS-SSS. Clostridium XIVb and Parasutterella were positively correlated with fecal propionate and propionate was negatively correlated with bloating. Furthermore, a direct negative correlation between Parasutterella and bloating was found. In the third network, several gut bacteria were positively correlated with fecal formate and isobutyrate or with isobutyrate only [Supplementary Figure 1C, http://links.lww.com/CM9/A10]. Fecal isobutyrate was negatively correlated with IFN-γ, and fecal formate was positively correlated with IL-10. A negative correlation was found between Parasutterella and IFN-γ.

Probiotics are frequently used to treat IBS, and although the mechanism of action is not clear, the approach has good efficacy. Many studies have revealed that probiotics can relieve abdominal symptoms including pain, bloating/distension, diarrhea and constipation. Recently, it has been demonstrated that 8 weeks of L. acidophilus, L. casei, and B. bifidum administration also has a beneficial effect on depression patients without IBS.[3] These studies suggest that probiotics may have promise for the treatment of patients with IBS with depression. Antidepressants are another widely used type of agent in the treatment of IBS, and prove to be effective.
It is thought that probiotics exert a positive effect on IBS by modulating gut microbiota. Some studies have shown alterations of the gut microbiota and function of metabolism after probiotic treatment. Some publications have also revealed that probiotics can influence the function of the gut microbiota at the transcriptional level. In the present study, the structure of the gut microbiota and the relative abundances of several bacteria were altered after probiotic treatment, and the ability of the gut microbiota to produce SCFAs was affected.

The therapeutic effect of antidepressants on IBS might be also partly caused by the ability to alleviate depression, which exaggerates abdominal symptom severity in IBS. Are the gut microbiota affected by antidepressants? The results of the present study showed that the profile of the gut microbiota shifted after treatment with Duloxetine, while the levels of plasma inflammatory cytokines decreased and the levels of fecal SCFAs were affected slightly. These changes could be caused by the effect of antidepressants on the central nervous system, leading to alteration of the interaction between the brain-gut axis and gut microbiota.

In addition, a significant negative correlation between fecal SCFA levels and abdominal symptom severity was found, suggesting that probiotics and antidepressants may affect IBS symptoms by influencing SCFA production via modulation of the gut microbiota. It is important to consider the fact that antidepressants have an effect on a patient’s appetite, and that diet is an important regulator of the gut microbiota. To exclude the effect of diet on gut microbiota, an elementary survey of the shift in the patients’ dietary structure was conducted, but no significant changes during the therapeutic period were found [Supplementary Figure 2, http://links.lww.com/CM9/A10]. Therefore, the alteration of the gut microbiota in the Duloxetine group was not likely caused by changes in diet.

Peripheral low-grade inflammation is considered as an essential pathophysiologic factor in both IBS and depression, our previous study found that the colon mucosal level of MCP-1 is upregulated in patients with IBS-D and depression.[1] In the present study, both Bifico and Duloxetine reduced the severity of abdominal symptoms and plasma levels of some pro-inflammatory cytokines (Bifico reduced the plasma levels of MCP-1 and IL-1B, while Duloxetine reduced the plasma level of MCP-1). A recent study also revealed that antidepressants have the potential to reduce systematic inflammation, [4] and this could be another means by which antidepressants alleviated depression and IBS symptoms. The present study suggest that it could also be that alterations of the gut microbiota induced directly by probiotics or indirectly via the brain-gut axis by antidepressants lead to decreases in peripheral inflammation because host immune function is affected by the gut microbiota to a large degree. Reduction of peripheral inflammation can alleviate abdominal symptoms, and the signal can be transmitted upward to the brain via the brain-gut axis, and even affect the function of the brain.

Gut microbiota influence host immunity, partly by activating pattern recognition receptors, but also through the production of SCFAs. Significant negative correlations between some gut bacteria and fecal SCFAs were detected in this study, in accordance with the results of previous studies. Furthermore, significant correlations between abdominal symptom severity and plasma cytokines levels were found. These important findings support the idea that the gut microbiota and fecal SCFAs influence abdominal symptoms through regulation of peripheral inflammation.

There are limitations of the present study, including samples size, gender distribution, and lacking of placebo control. As a pilot study, it still suggests that the interaction between the gut microbiota and the brain-gut axis playing part in the coexistence of IBS-D and depression, which may be further validated with big sample size. Growing evidence revealed that gender does influence the structure of gut microbiota, the present study was to see whether Bifico and Duloxetine treatment would alter the gut microbiota composition instead of comparing effects between those 2 agents, so gender differences might not have that much influence on our results. Our study was not performed in a blinded, placebo-controlled manner because of the ethical issue, though we know that the placebo effect is ubiquitous in the treatment of functional gastrointestinal disorders, with a rate of approximately 40%. [5] We found that 87.5% of patients who received Bifico and 100% of patients who received Duloxetine experienced improvements in IBS symptoms and depression. These rates are much greater than those expected to be caused by the placebo effect alone. In addition, as the designer of the IBS-SSS questionnaire recommended, a treatment should be considered effective when a reduction of >50 points of the IBS-SSS was obtained. In the present study, all patients experienced a reduction of score of >50. These findings, when taken together, may prove that the effects of the 2 treatments are genuine and not caused by the placebo effect.

The present study reveals that both probiotics and Duloxetine can alleviate abdominal symptoms and depression in patients with IBS-D. This may occur through regulation of peripheral inflammation via modulation of gut microbiota and fecal SCFA levels by the 2 agents, in turn, through the bidirectional regulation of the gut microbiota and the brain-gut axis. Further large-scale studies are warranted to confirm these findings.

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Conflicts of interest
None.

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