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Original Research

Investigation of the gut microbiota and adverse reactions associated with the combined bifidobacterium, lactobacillus, enterococcus and bacillus cereus tablets and rabeprazole in patients suffering from gastroesophageal reflux

Nannan Yin, Xuan Zhao, Lihong Shi

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Abstract

Objective: To explore the effect of proton pump inhibitor (PPI) Rabeprazole combined with Combined Bifidobacterium, Lactobacillus, Enterococcus and Bacillus cereus Tablets(CBLEB) in gastroesophageal reflux disease(GERD) on the regulation of gut microbiota and alleviation of gastrointestinal adverse reactions. **Methods:** A total of 60 patients with gastroesophageal reflux admitted to the Department of Gastroenterology of the Second Affiliated Hospital of Xuzhou Medical University from December 2022 to December 2023 were selected as the research subjects, and they were randomly divided into the Experiment group (rabeprazole + CBLEB) and the Control group (rabeprazole) by random number table method, and the general information and inflammatory factor levels of the two groups were compared, and the incidence of gastrointestinal adverse reactions and GerdQ score were compared between the control group and the experimental group. **Results:** The comparison of various data of subjects in the two groups showed that there were no significant differences in basic data such as age, body mass index and gender distribution between the control group and the experimental group. After treatment, bifidobacteria and lactobacillus in the experimental group were significantly increased in the control group, while GerdQ score in the experimental group was significantly lower than that in the control group, with statistical significance ($P<0.05$). The incidence of adverse reactions in control group was significantly higher than that in experimental group, the difference was statistically significant ($P<0.05$), CRP and GerdQ score were linearly correlated. **Conclusion:** CBLEB can improve the disturbance of gastrointestinal microbiota in GERD patients and alleviate gastrointestinal adverse reactions caused by PPI use.

Keywords: Gastroesophageal reflux; Probiotics; Rabeprazole; Gut microbiota

INTRODUCTION

Gastroesophageal reflux disease (GERD) is one of the common clinical diseases of the digestive system[1], which causes various symptoms and complications due to pathological retrograde entry of stomach contents into the esophagus[2]. Typical clinical manifestations are heartburn and regurgitation, accompanied by chest pain, epigastric pain, nausea and vomiting, indigestion, throat discomfort and cough[3,4]. In Western countries such as the United States and the United Kingdom, the incidence of GERD is higher (11-22%)[5], the epidemiological monitoring data of GERD in China showed that the incidence of GERD was 7.69% and showed an overall upward trend[6].

Proton pump inhibitors (PPIs) are acid-suppressing drugs widely used in acid-related diseases since their introduction in the 1980s[7]. They are the most effective first-line treatment for GERD patients. However, PPIs do not eradicate the disease[8]. Some patients may need to take the treatment for life as ongoing maintenance or when symptoms appear[9]. Although PPIs are rarely reported to have serious adverse reactions, long-term use of PPIs is still associated with adverse reactions, including gastrointestinal reactions, liver function damage, allergic reactions, kidney damage, infection[10]. Previous studies have reported a negative association between PPIs and pneumonia and mortality[11]. Among them, gastrointestinal reaction is the most common adverse reaction after the use of PPIs, mainly manifested as abdominal pain, diarrhea, constipation, nausea and so on. Recent studies have also shown, that long-term use of PPIs increases the risk of gut bacterial infections, such as Clostridium difficile, campylobacter, shigella and salmonella[12,13].

In recent years, more and more evidence shows that gut microbiota, as the “second genome” of the human body, is closely related to body health[14]. At the same time, studies have shown that GERD patients have gut microbiota disorders[15], and intestinal bacterial overgrowth, as an independent influencing factor, is closely related to the occurrence of GERD[16]. With the deepening of research, probiotics are a class of active microorganisms that can promote the ecological balance of host intestinal microflora and have beneficial effects on host health and/or physiological functions[17]. Their functions are mainly to improve the structure of gut microbiota, promote the

Nannan Yin. Bachelor of Pharmacy. Department of Pharmacy, Second Affiliated Hospital of Xuzhou Medical University, 536673615@qq.com
Xuan Zhao. Master of Medical Science. Department of Gastroenterology, Second Affiliated Hospital of Xuzhou Medical University xyefyzhaoxuan@163.com
Lihong Shi*. Associate Professor. Department of Gastroenterology, Second Affiliated Hospital of Xuzhou Medical University slh0186@126.com

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proliferation of beneficial bacteria in the intestine, and inhibit the growth of harmful bacteria, so as to improve the specific or non-specific immunity of the body[18]. probiotics have become an important means for immune regulation[17]. However, whether the variety and abundance of bacteria in the gastrointestinal tract of GERD patients can be regulated by probiotics, and whether the regulation of gastrointestinal adverse reactions in GERD patients has not been further studied. In this paper, we mainly observe the effects of CBLEB combined with rabeprazole on the gut microbiota of GERD patients and the changes in the incidence of adverse reactions.

MATERIALS AND METHODS

General information

A total of 60 GERD patients admitted to the Gastroenterology Department of the Second Affiliated Hospital of Xuzhou Medical University from June 2022 to June 2023 were collected and randomly divided into control group and experimental group. This study strictly follows the relevant medical research ethics requirements, and the possible risks and benefits of participating in this study are fully informed to the subjects by oral and paper documents. All subjects who meet the inclusion criteria must sign informed consent, and this study has been reviewed and approved by the Medical Ethics Committee.

Diagnostic Criteria

(1) Gastroscopy and pathological biopsy confirmed the presence of esophageal reflux caused inflammation or Barrett disease; (2) Abnormal reflux of acidic and/or alkaline substances in the esophagus was detected by 24 h esophageal pH monitoring; (3) After 1-2 weeks of continuous PPI treatment, the symptoms such as acid reflux and heartburn were significantly relieved. Meeting any of the above criteria can be diagnosed as GERD.

Detection of gut microbiota

The fecal samples of the two groups were detected before and after treatment, 1g samples were taken from each group, and the numbers of bifidobacterium, lactobacillus, yeast and enterococcus were counted by plate colony counting method, and expressed as the logarithmic value of 1g wet weight fecal colony number (lgCFU/g).

Gastroesophageal Reflux Disease Questionnaire (GerdQ questionnaire)

Subjects answered the following questions according to their own conditions in the past week, the frequency and severity of corresponding symptoms, and were guided by the gastroenterology medical staff to complete and calculate the score. Total score of 6 questions 8 points Consider GERD:

Treatment plan

The control group was treated with rabeprazole sodium enteric-coated capsule (Jichuan Pharmaceutical Group Co., LTD, Specification: 20mg, SinopOD H20061220), 20mg orally 30 minutes before meals, once a day. The experimental group was treated with the CBLEB based on the control group (Hangzhou Yuanda Biopharmaceutical Co., LTD., Specification :0.5g), 1.5g each time, 3 times a day. Both groups were treated for 8 weeks.

Statistical Analysis

SPSS 20.0 statistical software was used for data processing. The normality of measurement data was tested by S-W test (Shapiro-Wilk W test). Data with normal distribution were described by mean ± standard deviation (X±S), and T-test was used for inter-group analysis. P< 0.05 was a statistical difference, P<0.01 was a significant difference.

RESULTS

Basic patient information

Basic information of patients in the control group and the experimental group, including gender, Age and BMI, was summarized, and there were no significant differences between the two groups (*P*=0.7216, *P*=0.2307), as shown in Table 2.

Gut microbiota changes

Before treatment, there was no significant difference in the mean value of fecal flora detection between control group and experimental group(P>0.05). After treatment, bifidobacterium(*P*=0.0184) and lactobacillus(*P*=0.0097) in the experimental group were significantly higher than those in the control group, with statistical significance (**P*<0.05 vs control group). There was no significant difference between yeast and enterococcus compared with control group (P>0.05). Table 3.

Gastroesophageal reflux score

Before treatment, GerdQ scores in both groups were higher than the standard value, and there was no significant difference between the two groups (Table 4). After treatment, scores were significantly decreased compared with those before treatment (*P*<0.01), and the differences were statistically significant (*P*<0.05vs control group). The experimental group was slightly lower than the control group, but there was no statistical difference , as shown in Table 4.

Inflammatory factors

Compared the levels of inflammation-related factors before and after treatment, IL-1β, IL-6 and TNF-a were all lower than the critical value before and after treatment, CRP was higher than the normal critical value before treatment. After treatment, the indexes of subjects in both groups were decreased, and the CRP decrease trend of the experimental group was more obvious than that of the control group((*P*=0.0486)), with a significant difference (*P*<0.05 vs control group), as shown in Table 5.

Correlation analysis between GerdQ score and inflammatory factors

The analysis of inflammatory factors showed that the CRP level of the experimental group after treatment was significantly lower than that before treatment. We further analyzed the correlation between CRP and GerdQ score in the experimental group. We did linear regression analysis of the two groups of data before and after treatment in 30 subjects, and the results showed that there was a linear correlation between CRP values

Table 1. The GerdQ questionnaire respondents enter the frequency scores after reflecting on their symptoms over the previous week

Question	Frequency score/d	points
1. How often did you have a burning feeling behind your breastbone (heartburn)?	0	0
	1	1
	2-3	2
	4-7	3
2. How often did you have stomach contents (liquid or food moving upwards to your throat or mouth (regurgitation)?	0	0
	1	1
	2-3	2
	4-7	3
3. How often did you have a pain in the centre of the upper stomach?	0	3
	1	2
	2-3	1
	4-7	0
4. How often did you have nausea?	0	3
	1	2
	2-3	1
	4-7	0
5. How often did you have difficulty getting a good night's sleep because of your heartburn and/or regurgitation?	0	0
	1	1
	2-3	2
	4-7	3
6. How often did you take additional medication for your heartburn and/or regurgitation, other than what the physician told you to take? (such as Tums, Rolaids, Maalox?)	0	0
	1	1
	2-3	2
	4-7	3

Table 2. Basic data of the two groups of patients

Group	n	age	sex (male/female)	BMI
Control	30	63.73±12.74	17/13	23.17±1.71
Experiment	30	57.92±10.11	14/16	23.59±2. 17

Table 3. Changes of gut microbiota in two groups before and after treatment ($lg^{CFU/g}$)

Group	(n)	Bifidobacterium		Lactobacillus		Saccharomyces		Enterococcus	
		Before	After	Before	After	Before	After	Before	After
Control	30	6.6	8.4	6.3	7	5.6	6	5.9	6.5
Experiment	30	6.3	9.2	6	7.6	5.3	6.2	6.1	6.7

Table 4. The GerdQ scores of the two groups before and after treatment

Group	n	Before	After	*P*
Control	30	10.4	6.43	*P*<0.01
Experiment	30	10.57	6.53	*P*<0.01

Table 5. Comparison of inflammatory indexes before and after treatment

Group	(n)	IL-6(pg/ml)		IL-1β(pg/ml)		TNF-α(pg/ml)		CRP(pg/ml)	
		Before	After	Before	After	Before	After	Before	After
Control	30	3.46	1.49	2.7	1.53	2.4	1.58	8.92	3.73
Experiment	30	3.17	1.54	3.15	1.45	1.97	1.62	10.23	3.54

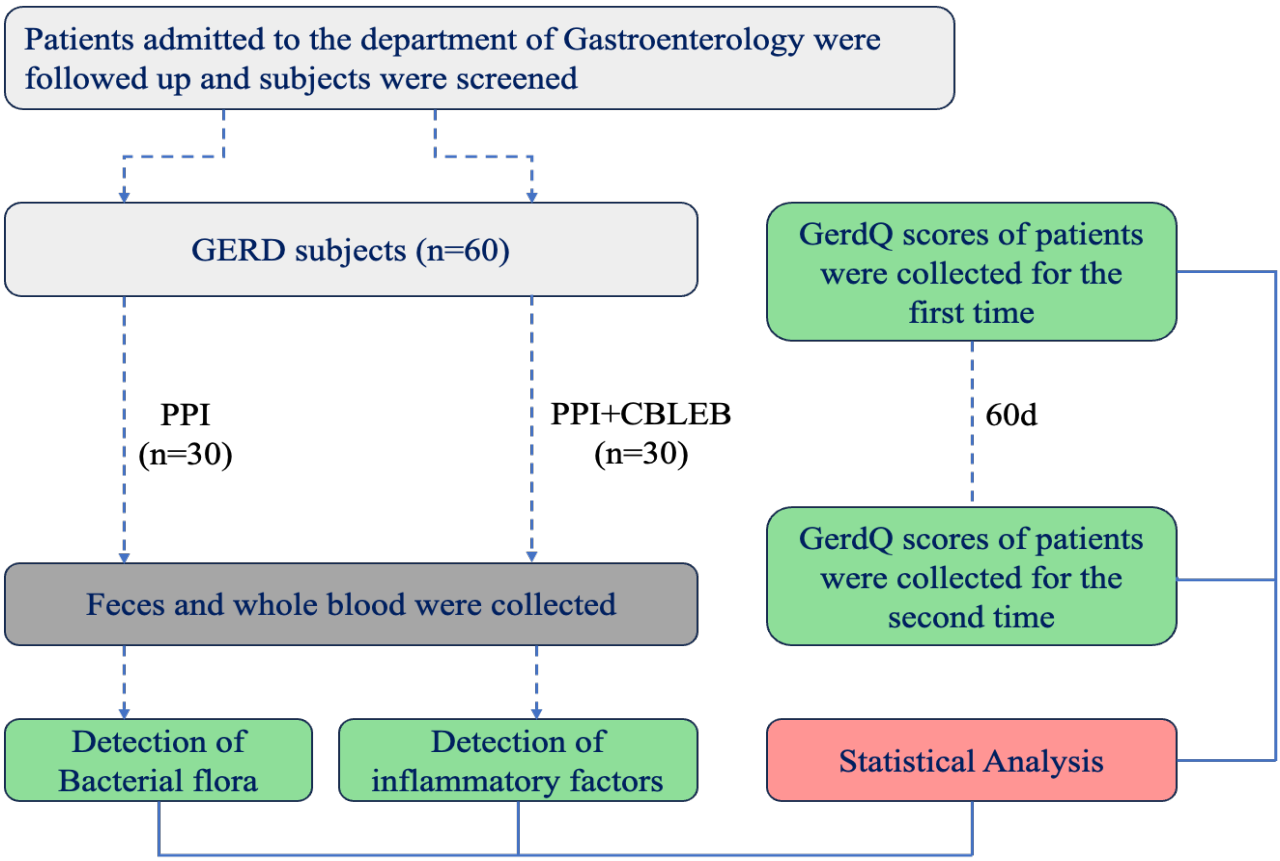

Figure 1. The study design was complemented by a flow chart illustrating the screening, recruitment, and randomization of participants to the intervention group.

and GerdQ scores (R^2=0.65), as shown in Figure 2.

Incidence of adverse reactions

The incidence of adverse reactions, including inflammatory bowel disease, abdominal pain, and loss of appetite, were reported in the two groups. Compared with the control group, the incidence of adverse reactions in experimental groups was decreased, and the total incidence was significantly decreased (*P<0.01 vs control group), as shown in Table 6.

DISCUSSION

GERD is one of the common digestive diseases in clinic, and its pathogenesis is relatively complex[19]. PPIs are commonly prescribed antacid medications for treating acid-related disorders[20], it also be a potential therapeutic agent for Pulmonary Fibrosis[21], and PPIs significantly increased the risk of reaching the composite endpoint in COVID-19 patients[22]. Although PPIs are the first choice for the treatment of gastroesophageal reflux disease, 4-8 weeks of PPI therapy should be the basic therapy for GERD patients[23]. But there is still a subset of people treated with PPI whose symptoms are poorly controlled[24]. Rabeprazole is a commonly used proton pump inhibitor[25]. By acting on gastric mucosal parietal cells, rabeprazole can inhibit the intracellular tubular vesicles and secreted microtubules of gastric parietal cells, thereby controlling the secretion of basic gastric acid and gastric acid secretion caused by external stimulation[26]. By inhibiting pyroptosis in gastric epithelium, rabeprazole inhibits inflammatory reactions[27].

Recent studies have shown that long-term application of PPIs to control the secretion of gastric acid in the body will, to a certain extent, lead to the decline of gastric acid barrier function[28], further cause gut microbiota disorders, and then

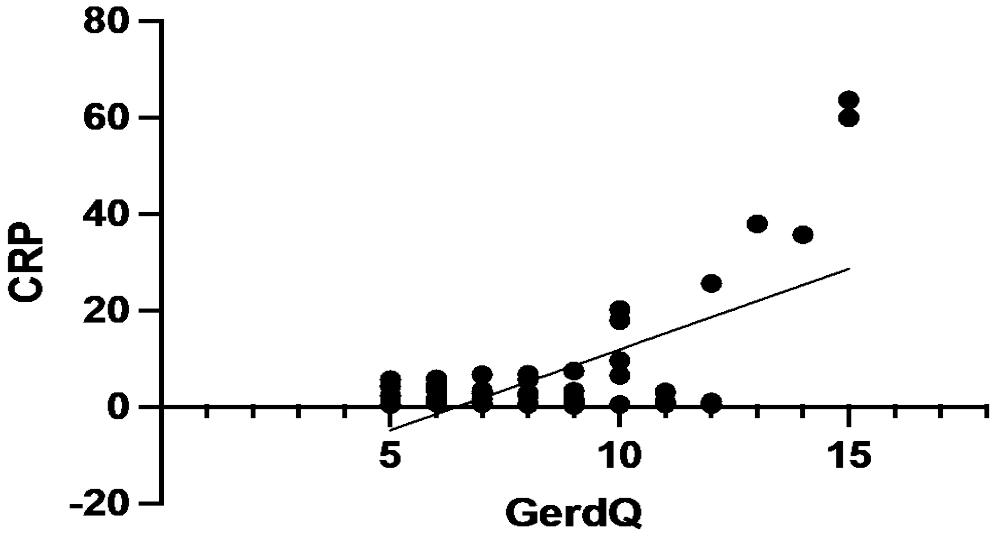

Figure 2. TCorrelation analysis of GerdQ score and CRP

Table 6. Incidence of adverse reactions in the two groups (%)

Group	n	Enteritis	Abdominal pain	Loss of appetite	Total Rate
Control	30	6.6	3.3	6.6	16.6
Experiment	30	3.3	0	3.3	6.6

lead to secondary infections and other adverse reactions[29]. A study of gastric ulcer showed that PPIs had a regulatory effect on serum gastroenterokinase expression and serum oxidative stress injury[26], however, it has not been reported in GERD patients. This study found that rabeprazole could significantly improve gastroesophageal reflux performance and reduce GerdQ score in GERD patients. However, the control of adverse reactions was poor.

The gut microbiota is composed of a large number of microorganisms residing in the gut, which can be divided into harmful flora, neutral flora and beneficial flora according to their different functions and effects, as well as their effects on the human body[18]. Gut microbiota is now recognized as one of the key factors contributing to the regulation of host health. Based on the development of molecular tools and techniques (metagenomics, metabolomics, lipidomics, subtranscriptomics), the complex interactions between the body and different microorganisms are gradually becoming a research focus[30]. The link between the gut microbiome and many diseases, such as GERD, is being unraveled[31]. Under normal conditions, various gut microbiota maintain a relatively balanced state, and beneficial bacteria such as bifidobacterium and Lactobacillus account for about 90% of the total flora. The superorganisms composed of them jointly play the roles of nutrition and material metabolism, immune regulation and biological barrier, and the destruction of their balance is easy to cause related diseases. One study showed that PPI use is associated with an increased risk of serious infections in young children[10], and this process is primarily achieved by regulating the gut microbiota. Studies have also shown that taking PPIs increases genes involved in bacterial invasion[32]. Therefore, it is of great significance to maintain gut microbiota homeostasis during PPI use. In this study, it was found that the use of bifidobacterium tetrad viable tablets combined with rabeprazole could significantly improve the imbalance of gastrointestinal flora, reduce gastrointestinal adverse reactions, improve the clinical application expectation of rabeprazole in GERD, and improve the medication compliance of patients.

In this study, patients with Helicobacter pylori infection were excluded from our study. Studies have shown that PPIs are more effective in people with H. pylori infection than those without[33,34]. Therefore, for the patients with gastroesophageal reflux complicated with Helicobacter pylori infection, whether rational use of probiotics can promote the improvement of the disease course of the patients is worthy of further study.

CONCLUSION

In summary, the combination of rabeprazole and bifidobacterium quadruplex viable tablets for GERD patients can significantly reduce the incidence of adverse reactions, improve the balance of gut microbiota, and improve medication safety and compliance of patients. However, the mechanism of its joint regulation has not been discussed, which is worth further study.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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