BuPiHeWei Decoction Ameliorate 5-Fluorouracil-Induced Intestinal Dysbiosis in Rats by Regulating the Treg/Th17 Signaling Pathway

Sun Zhigao, Hu Yazhuo, Wang Yuguo, Feng Jian, Dou Yongqi

Sun Zhigao, Department of Traditional Chinese Medicine, Chinese PLA General Hospital, Beijing 100853, China & Department of Traditional Chinese Medicine, Hainan Branch of Chinese PLA General Hospital, Hainan 572013, China

Hu Yazhuo, Institute of Gerontology, Chinese PLA General Hospital, Beijing, 100853, China

Wang Yuguo, Feng Jian, Dou Yongqi, Department of Traditional Chinese Medicine, Chinese PLA General Hospital, Beijing 100853, China.

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Corresponding to: Yongqi Dou, Department of Traditional Chinese Medicine, Chinese PLA General Hospital, Beijing 100853, China. dyqi_301@yeah.net

Telephone: +86-010-66939456

Abstract

OBJECTIVE: To observe the effect of BuPiHeWei (BPHW) decoction on diarrhea and Intestinal flora disorder induced by 5-fluorouracil (5-FU), and to explore the intervention mechanism to provide experimental evidence for clinical treatment.

METHODS: Thirty-five male Sprague-Dawley rats were randomly divided into four groups: normal control group, 5-FU group, 5-FU+Bacillus Licheniformis Capsule group (0.2g/kg for five
consecutive days), 5-FU+BPHW decoction group (10.5g/kg for five consecutive days). Animal models were established by intraperitoneal injection of 5-FU (30 mg/Kg for five consecutive days). By the end of the treatment period, diarrhea score and the change of the intestinal flora was examined by 16S rDNA high-throughput sequencing. The expression of IL-10, IL-17, IL-21, Foxp3 and RORγt in the small intestine were detected with qPCR and Western blot method. Furthermore, the contents of TNF-α, IL-1β, IL-6 and TGF-β in the small intestine was assessed by ELISA.

RESULTS: In this study, BPHW decoction could effectively reduce diarrhea score, increase the content of Bacteroidetes and Prevotellaceae-Alloprevotella, reduce the proportion of Proteobacteria, Escherichia-Shigella, Ruminococcaceae_NK4A214 and Ruminococcaceae-UCG-005 in the rat intestine after 5-FU chemotherapy. In addition, BipuHewei decoction could significantly suppressed the expression of IL-10, IL-17, IL-21, Foxp3, RORγt and the contents of TNF-α, IL-1β, IL-6 and TGF-β in the small intestine.

CONCLUSION: Our findings suggested that BuPiHeWei decoction could promote intestinal immune balance and attenuate intestinal inflammatory by regulating Treg/Th17 associated factors, thereby playing a regulatory role in intestinal dysbiosis after chemotherapy.

Keywords: BuPiHeWei decoction, 5-FU, chemotherapy, intestinal flora, Treg/Th17, mechanism

INTRODUCTION

Chemotoxicity, such as diarrhea and vomiting is considered to be associated with intestinal
dysbiosis after chemotherapy. Among them, the abnormal differentiation of T cell caused by chemotherapeutic drugs is characterized as the critical factor, which subsequently promotes the abnormal increase of downstream inflammatory factors, including TNF-α and IL-1β.

In recent years, T helper cells (Th17) / T regulator cells (Treg) have been found to maintain T cell immune homeostasis through various pathways, thereby playing an important role in regulating the balance body inflammatory factors. As a central medium for the production of pro-inflammatory cytokines, Th17 cells exert pro-inflammatory effects by releasing IL-17 and other cytokines to up-regulate inflammatory factors (including downstream IL-1β, TNF-α) in a cascade-release pattern. Treg exerts a dual effect, which can not only induce immune tolerance, attenuate immune defense response of Th1, but also promote Th17 development, aggravate the body's inflammatory damage. Excessive inflammatory mediators can cause changes in the total amount and structure of the intestinal flora and even flora translocation by damaging the intestinal homeostasis (via interfering with macrophages, NK cells, and mucosal epithelial barriers). Therefore, it is clear that Th17 and Treg play an important role in maintaining the body's immune balance, and the induced intestinal immune dysfunction may be the key to intestinal dysbiosis after chemotherapy.

Microecological agents are commonly used in clinical treatment of intestinal dysfunction. Studies have shown that they can increase probiotics in vivo and inhibit the reproduction of pathogenic bacteria, thereby alleviating clinical symptoms such as diarrhea and constipation. The pathogenesis of intestinal microecosystem disorder after chemotherapy is complex. Intestinal microecosystem preparations for the treatment by supplementing one or several probiotics, Although some effects have been achieved, they cannot significantly reduce the
incidence of gastrointestinal toxicity and side effects after chemotherapy.

BPHW decoction is a commonly used traditional Chinese medicine therapeutics after chemotherapy, which has multi-target regulation effects and can effectively alleviate gastrointestinal adverse reactions such as diarrhea, constipation and vomiting. Relevant studies have shown that it can effectively regulate the expression of intestinal probiotics and pathogenic bacteria.[8] Nevertheless, it is still unclear whether the mechanism of BPHW decoction in interfering intestinal dysbiosis is mediated by the Th17 and Treg pathway.

In this study, intraperitoneal injection of 5-Fu was used to induce the intestinal dysbiosis in rats. By detecting the diarrhea score, fecal microbiota and expression of Th17/Treg-related factors in small intestine, we aimed to investigate the characteristics of post-chemotherapy intestinal dysbiosis and the mechanism of BPHW decoction intervention, and provide reliable bases for clinical application by comparison with microecological preparations.

MATERIALS AND METHODS

Rats

Thirty-five male Sprague-Dawley rats (weight 200±10g) were provided by the Experimental Animal Center of the 302 military Hospital [animal certificate number: SYXK (Army)-2012-0010]. All rats were housed on a 12 h light/dark cycle in a temperature- and humidity-controlled room and maintained on a standard diet and water ad libitum. Animal experimental procedures were conducted in accordance with the Guidance Suggestions for the Care and Use of Laboratory Animals, formulated by the Ministry of Science and Technology of China and the protocols were approved by the Animal Ethics Committee at
Preparation of BPHW Decoction

BPHW decoction was composed of astragalus (20g), atractylodes (15g), clam shell (10g), ginger pinellia (10g), divine song (15g), malt (15g) and alfalfa (15g). Herbs were provided and identified by the Chinese Pharmacy of Chinese PLA General Hospital (Beijing, China). The medicine was mixed, added with 1.5-fold volume of water, immersed twice, mixed and filtered, concentrated in a constant temperature water bath at 80 °C to a crude drug containing 5 g / ml of the drug solution, which was stored at 4 °C for further application.

Reagents and instruments

TRIzol®RNA extraction kit was purchased from Sigma (Sigma, California, USA); High-Capacity cDNA Reverse Transcription Kits (Cat.NO.4368814) was purchased from Thermofisher (Thermofisher, Waltham MA, USA); Real-time PCR kit LightCycler® 480 SYBR Green I Master (CAT.NO.04707516001, U.S.A) was purchased from Roche Applied Science (Roche, Basel, Swiss). RORγt antibody was purchased from Abcam (Abcam, Inc, U.S.A). FOXP3 antibody was purchased from Abcam (Abcam, Inc, U.S.A).

The following instruments were used in this study: real-time PCR amplifier (Applied Science 384, Basel, Swiss); Gradient PCR amplifier (T100, Bio-rad, USA), Library construction kit (Ion Plus Fragment Library Kit 48 rxns, Thermo Scientific, USA); High throughput sequencing analyzer (IonS5TMXL, Thermofisher, USA); microplate reader (multiskcan FC, Thermofisher, USA); electrophoresis apparatus (Powerpac basic, Bio-Rad, USA).

Model establishment and group processing
Random number method was utilized to divide all rats into blank control group (N = 8), model group (M = 9), Bacillus Licheniformis Capsule group (BLC, N = 9), BPHW decoction group (BPHW, N = 9). Rats in the blank control group were intraperitoneally injected with the same volume of normal saline, rats in the other groups were intraperitoneally injected with 5-Fu (10ml: 0.25g, Shanghai Xudong Haipu Pharmaceutical Co., LTD.) at 30mg/(Kg·d) for five consecutive days to establish chemotherapeutic rat model.[9]

Rats in the blank control group and model group were gavaged with 2 ml of normal saline daily on the day of model establishment (for five consecutive days). Rats in the BLC group were gavaged with 0.2 g/kg BLC (2 ml, dissolved in normal saline) daily (for five consecutive days). Rats in the BPHW group were gavaged with 10.5g/kg BPHW decoction daily (for five consecutive days). The dose was equivalent to adult dose. On the sixth day (24 hours after the final gavage), 0.5 g of feces from rats of each group was reserved in sterile EP tubes, which was stored at -80°C for further use. Finally, all rats were executed with spinal dislocation, and the small intestine tissues were removed from rats and reserved in -80°C for further experiments.

**Diarrhea score**

The defecation of rats in each group was observed before and after the experiment, and the diarrhea score was made according to AKinobu Kurital method,[10] (Table 1).

<table>
<thead>
<tr>
<th>Degree of diarrhea</th>
<th>Symptoms</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>normal</td>
<td>normal stool or absent</td>
<td>0</td>
</tr>
</tbody>
</table>
slight  slighty wet and soft stool 1
moderate  wet and unformed stool with moderate perianal of the coat 2
severe  watery stool with severe perianal staining of the coat 3

High-throughput sequencing

Five samples were randomly selected from each group for detection. The genomic DNA of the samples were extracted using the CTAB method, and the sequencing library preparations and high-throughput sequencing were conducted at Novogene, Inc. (Beijing, China). Briefly, 30–50ng DNA was used to generate amplicons through V3 and V4 hypervariable regions of prokaryotic 16S rDNA. The sequences of forward primer for the v3 and V4 region used in this study were as follows “CCTAYGGGRBGCASCAG” and reverse primers containing the sequence “GGACTACNNGGGTATCTAAT”. The Ion Plus Fragment Library Kit (Thermo Scientific, USA) was used to construct the Library and the high-throughput sequencing were conducted after quantification by Qubit 2.0 Fluorometer.

RNA Extraction and qPCR

According to the gene sequence in Genbank database, Primer 5.0 software was used to design primers (Table.2). Total RNA was extracted according to manufacturer’s instruction, followed by micro-ultraviolet spectrophotometer (NanoDrop2000, Thermo Scientific) to determine the concentration and purity of RNA. Afterwards, RNA was reversely transcribed into cDNA according to the manufacturer’s instructions. The PCR reaction mixture (10 μL) consisted of 5μL 2 × Master Mix (PCR polymerase), 0.5μL Forward primer, 0.5μL Reverse primer, 1μL cDNA template, 3μL RNase-free water. We used the two-step PCR reaction
conditions: 95 °C denaturation for 5 min, 40 cycles under the following conditions: denaturation at 95 °C for 10 s, annealing at 55 °C for 15 s, extension at 72 °C for 20 s. The SYBR green fluorescent signals were acquired at 72 °C. Standard curves were constructed from PCR reactions using 10-fold serial dilutions of known bacterial DNA. The relative gene expression was converted and shown as fold changes (\(=2^{\Delta\Delta C_T}\)).

Table 2  Primers used for quantitative Real-time PCR

<table>
<thead>
<tr>
<th>Genes</th>
<th>Forward primer</th>
<th>Reverse primer</th>
</tr>
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<tbody>
<tr>
<td>GAPDH</td>
<td>5'-GGTGAAGGTGCTCGTGTAACG-3'</td>
<td>5'-CTCGCTCTGGAGATGGTG-3'</td>
</tr>
<tr>
<td>IL17a</td>
<td>5'-CTGGAGGCTACAGTGAAG-3'</td>
<td>5'-TGAAGTGAACGGTTGAG-3'</td>
</tr>
<tr>
<td>IL-10</td>
<td>5'-AGAAGGACCAGCTGACAAC-3'</td>
<td>5'-GCATCCTTCTTACCAGG-3'</td>
</tr>
<tr>
<td>IL-21</td>
<td>5'-CAAGCCATCAACACTGGAAAC-3'</td>
<td>5'-TTCTCATACAAATCACAGGAAG-3'</td>
</tr>
<tr>
<td>RORγt</td>
<td>5'-GTCCAGAGATGCTGTCA-3'</td>
<td>5'-GGAGTCTTGCTTGGTGT-3'</td>
</tr>
<tr>
<td>Foxp3</td>
<td>5'-ATGTTGCTACTTCAGA-3'</td>
<td>5'-CCTTCTCCTCCACTC-3'</td>
</tr>
</tbody>
</table>

Western Blot Analysis

Otal protein was extracted by using protein lysate according to the relevant protocols, followed by protein quantitation via BCA method. The above supernatant (containing about 80 μg of protein) was subjected to 12% SDS-PAGE, and subsequently transferred to nitrocellulose (NC) filter membrane. The blots were probed with appropriate antibodies against Foxp3, ROR γ t, β-actin, followed by incubation with secondary antibodies conjugated with horseradish peroxidase (HRP; Thermo Fisher, Waltham, MA, USA). Millipore ECL (Millipore, Massachusetts, USA) was used for chemiluminescence, and analyzed by using grayscale value with image lab software (BioRad, Hercules, CA, USA).

ELISA Assay for TNF-α, IL-1β, IL-6 and TGF-β in small intestine mucosa
According to the manufacturer’s instructions, the levels of TNF-α, IL-1β, IL-6 and TGF-β in small intestinal tissues were determined by ELISA method. The microporous plate spectrophotometer was used to measure the optical density at 450 nm, and the calculated results were based on the standard curve.

Statistical analysis

16S rDNA data were analyzed by QIIME package data (QIIME, Colorado, USA) and R programming language (R, Auckland, New Zealand). MetaStat method was utilized for differential difference of intestinal species between groups, where a P < 0.05 was considered as statistically significant. SPSS17.0 software package (SPSS, Chicago, IL, USA) was used for the statistical analysis of the rest of the data. In brief, measurement data with normal distribution and homogeneity were shown as $\bar{x} \pm s$ and analyzed by ANOVA. While, data without normal distribution or homogeneity were analyzed by rank sum test. A P-value < 0.05 was considered statistically significant.

RESULTS

The effect of BPHW Decoction on the diarrhea score of 5-Fu simulated chemotherapeutic rats

The diarrhea scores of all groups before the experiment were 0 (P > 0.05) and there was no symptoms of diarrhea occurred in the normal control group during the experiment. Compared with the normal control group, the diarrhea index of the model group was significantly increased on the 6th day (P < 0.01). Compared with the model group, the diarrhea scores of the rats in the BPHW group and the Bacillus Licheniformis Capsule group were significantly
reduced (P<0.01, P<0.05). There was no significant difference between the BPHW group and the Bacillus Licheniformis Capsule group, but the average score of diarrhea was lower.

(Fig.1 A,B).

![Graphs](image)

Fig 1  Comparison of diarrhea scores among different groups
*indicated p< 0.05 and **indicated p< 0.01 between groups

Results of 16S rDNA high-throughput sequencing of intestinal flora

A total of 42796 valid sequences were obtained from 20 samples, and the average length of the sequences was 409-424 bp. After leveling, the OTUs at 97% of the similar levels was statistically analyzed for bioinformatics, which gave rise to a total of 1257 OTUs. Each type of OTUs can be approximately considered as one species.

Taxonomic analysis showed that 20 samples contained a total of 18 phylums. In terms of number. In the normal control group, the proportion of Firmicutes was the highest and Firmicutes, Bacteroidetes and Proteobacteria were the dominant bacteria (Fig. 2). Compared with the normal control group, there was a decreasing trend (62.7% vs 46.5%) of Firmicutes in the model group (62.7% VS 46.5%), without statistical significance (P>0.05). In addition, Bacteroidetes was significantly increased (34% VS 10.4%, P< 0.05) while Proteobacteria was
significantly decreased (1.1% VS 41.8%, P < 0.01) in the model group. BPHW decoction could effectively reverse the above-described tendency in the model group, as indicated by significantly increased content of Bacteroidetes (46.9%) and decreased content of Proteobacteria (11.9%). In spite of no statistical significance with BLC group, the recovery tendency was more obvious in the BPHW group (P>0.05). (Fig. 3)

Further analysis was utilized to analyze the strain changes in each group from the species level. By comparison with the SILVA database, 201 strains were detected from the 20 samples. The intestinal flora of the different samples was significantly different, indicating the diversity of intestinal flora (Fig. 2). Compared with the normal control group, the Escherichia-Shigella (0.1% VS 36%), Ruminococcaceae_NK4A214 (0.8% VS 6.1%) and Ruminococcaceae-UCG-005 (0.5% VS 2.1-%) were significantly increased (all P < 0.05), while Prevotellaceae-Alloprevotella was significantly decreased (3% VS 0.2%, P < 0.05) in the model group. Lactobacillus has been reported to be significantly altered in previous studies, here, however, we failed to reveal any statistical significance (17% VS 12%, P > 0.05). BPHW decoction could significantly decrease the content of Escherichia-Shigella (9.2% VS 36%, P < 0.05). Additionally, compared with the model group, BPHW decoction led to obviously decreased content of Ruminococcaceae_NK4A214 (0.8% VS 6.1%), Ruminococcaceae-UCG-005 (1.2% VS 2.1%) and obviously increased content of Prevotellaceae-Alloprevotella (0.5% VS 0.2%), but not statistically significant (Fig. 4).
Figure 2 The relative abundance of fecal species in each group of rats

Phylum level in the left figure, and genus level in the right figure.

Figure 3 The differential comparison of fecal species in each group of rats (at phylum level)

*indicated p < 0.05 and **indicated p < 0.01 between groups.
Figure 4 The differential comparison of fecal species in each group of rats (at genus level)

*indicated $p<0.05$ and **indicated $p<0.01$ between groups

The effects of BPHW Decoction on the mRNA expression of IL-17, IL-10, IL-21, ROR $\gamma$ t and Foxp3 in the small intestine of 5-Fu simulated chemotherapeutic rats

As shown in Fig. 5, compared with the normal group, the expression levels of IL-17, IL-10, IL-21, ROR $\gamma$ t and Foxp3 were significantly increased in the small intestine of the model group ($P<0.05$). In addition, BPHW Decoction could effectively regulate the immune balance by decrease the content of IL-17, IL-10, IL-21, ROR $\gamma$ t and Foxp3, which was significantly improved compared with those in the model group ($P<0.05$).
Figure 5 The gene expression of IL-17, IL-10, IL-21, ROR γ t and Foxp3 in the small intestine of rats in each group

* P<0.05 and ** P<0.01 versus Normal group. *P<0.05 and **P<0.01 versus Model group. △ P<0.05 versus BLC group.

The effect of BPHW Decoction on the protein expression of ROR γ t and Foxp3 in the small intestine of 5-Fu simulated chemotherapeutic rats

As shown in Fig. 6, compared with the normal control group, the expression of RORyt and Foxp3 was significantly increased in the small intestine of the model group (P<0.01). BPHW
Decoction could effectively regulate the immune balance by suppressing the expression of RORγt and Foxp3, which was improved compared with the model group (P<0.05, P<0.01).

![Figure 6 The protein expression of RORγt and Foxp3 in the small intestine of rats in each group. *indicated p<0.05 and **indicated p<0.01 between groups.]

Effect of BPHW Decoction on the contents of TNF-α, IL-1β, IL-6 and TGF-β in intestinal mucosa of rats treated with 5-FU chemotherapy

The results showed that: Compared with the normal control group, the contents of TNF-α, IL-1β, IL-6 and TGF-β in the small intestine of rats in model group, BPHW group and BLC...
group increased significantly ($P<0.01$, $P<0.05$). The levels of TNF-α, IL-1β, IL-6 and TGF-β in BPHW group and BLC group were significantly lower than those in model group ($P<0.01$, $P<0.05$). There was no significant difference between BPHW group and BLC group ($P>0.05$). (Fig.7A,B,C,D).

![Figure 7](image-url)

Figure 7. Effect of BPHW Decoction on the contents of TNF-α, IL-1β, IL-6 and TGF-β in intestinal tissue of rats after 5-FU chemotherapy

$^\ast\ast P<0.01$ versus Normal group. $^\#P<0.05$ and $\^\#P<0.01$ versus Model group.

**DISCUSSION**

Patients undergoing chemotherapy are often accompanied with gastrointestinal side effects, such as diarrhea and vomiting, which is generally considered to be closely associated with intestinal dysbiosis after chemotherapy.\(^\text{[11]}\) The normal intestine is dominated by Firmicutes and Bacteroidetes.\(^\text{[12]}\) Chemotherapeutic drugs can lead to significantly decreased total
amount of intestinal flora, especially the probiotics classified in the anaerobic genus, while the number and the proportion of pathogenic bacteria both increase during chemotherapy. For example, methotrexate (MTX) could decrease the total number of intestinal flora by approximately 29.6% in children with leukemia, moreover, the number of bifidobacteria, lactobacilli and E. coli was significantly decreased during chemotherapy. BEAM regimen could increase the amount of Escherichia coli and Bacteroides, while significantly decrease the amount of E. faecalis and Bifidobacteria in the intestine of patients with non-Hodgkin's lymphoma. Although different chemotherapeutic drugs exert certain effects on the intestinal flora, 5-FU and irinotecan are the most common, such as obvious chemotherapy-induced diarrhea (CID), which is thought to be associated with intestinal dysbiosis caused by the above-mentioned chemotherapeutic drugs.

In traditional Chinese medicine, intestinal dysbiosis caused by chemotherapeutic drugs is due to the dysfunction of transportation and transformation in spleen. And BPHW Decotion is an effective approach to prevent and treat intestinal dysbiosis after chemotherapy in clinical practice. Hai Yanjie et al. found that Jianpi Shenshi Decoction could increase the proportion of bifidobacteria and lactobacilli in patients with advanced colorectal cancer who underwent chemotherapy, which had a regulatory effect of intestinal flora, with the total effective rate of 86.7%. Wang Zhuo et al. confirmed that the spleen-deficient rats had disorder and dysbiosis of intestinal flora by diversity analysis of ERIC-PCR fingerprint, and Sijunzi decoction could significantly improve the intestinal flora diversity in the spleen-deficient mice, especially for the recovery of bifidobacterium and lactobacillus.

Recent studies have found that intestinal flora and intestinal immune barrier interact with each
other. On the one hand, colonization of intestinal probiotics promotes the formation of intestinal immune function. In artificially reared germ-free mice, T cells and immune factors were deficient, the number of dendritic cells in lymph nodes of intestinal mucosa lamina propria decreased, and the content of sIgA and antimicrobial peptide decreased. The low antibody level of aseptic mice indicates that intestinal flora plays an important role in promoting intestinal immune system. On the other hand, the intestinal immune barrier function plays an important role in regulating the normal development and maintenance of intestinal microecology. The intestinal immune system secretes many immune factors to make intestinal microorganisms distribute regularly in different layers in the intestinal cavity, inhibit the excessive reproduction of harmful bacteria, and maintain the intestinal microecological balance. It has been reported that the absence or dysfunction of SIgA and HD will lead to the loss or mutation of activation-inducible cytidine deaminase, thus inhibiting the intestinal immune function, leading to the proliferation of harmful bacteria and the imbalance of flora. The mechanism of the effect of chemotherapeutic drugs on intestinal immune barrier is complex. In recent years, with the clarification of the role of Th17/Treg in the intestinal immune hub, regulating Th17/Treg balance has become a research hotspot in regulating intestinal immune balance. Treg is a subset of CD4+CD25+ T cells that can act as negative regulators. Foxp3 is the main regulator of Treg, which promotes the secretion of inhibitory cytokines such as IL-10 by Treg, thereby playing an important role in the negative regulation of body immunity. Th17 cells are the central mediator of pro-inflammation, and RORγt is the signature factor of Th17 cells. IL-17, as its main effector molecule, exerts a strong pro-inflammatory effect and can induce the secretion of IL-6, IL-21, IL-22 and TNF-α,
subsequently producing a strong inflammatory response and causing tissue damage.\textsuperscript{[24]} In addition, Th17 can recruit and activate neutrophils by inducing CXC chemokines via the MAPK and the NF-κB pathway to exert its biological effects.\textsuperscript{[25]}

Treg can affect the expression of Th1, Th2 and Th17 cells and their immune balance.\textsuperscript{[26-27]} For example, an increase in the content of Treg cells can attenuate the expression of INF-γ, thereby inhibiting the bactericidal ability of macrophages.\textsuperscript{[28]} On the contrary, it drives Th2-type immune responses and induces immune tolerance.\textsuperscript{[29]} Treg has a dual role in the Th17 expression. It is generally believed that Treg exerts an antagonistic effect on Th17 cells, which mainly depends on the common regulation of TGF-β1 and IL-6.\textsuperscript{[30]} On the other hand, Treg can promote Th17 transformation, which could not only promote the preferential expression of Th17 cells via the suppression of Th1 and Th2 factors, but also promote the differentiation into Th17 cells via itself.\textsuperscript{[31]} Studies have revealed that Th17 and Th1 cells have the same cell surface markers, which may be derived from the same precursor cells, and they could differentiate according to the cytokines in the environment, and could be transformed into each other.\textsuperscript{[32]} In the process of inflammation, the transformation of Th17 cells into Th1 cells might be beneficial to prevent tissue damage caused by excessively invasive inflammatory responses during pathogen clearance.\textsuperscript{[33]} Taken together, the imbalance of Treg/Th17 could induce the drift from Th1 to Th2 and aggravate the Th17-mediated inflammatory response, thereby interfering with the body immune balance.\textsuperscript{[34]}

The results of this experiment showed that the diarrhea severity score of the model group was significantly higher than that of the normal control group and there was a tendency of decreased Firmicutes and Bacteroidetes, while significantly increased Proteobacteria.
Additionally, in terms of species level, Escherichia-Shigella, Ruminococcaceae_NK4A214 and Ruminococcaceae-UCG-005 were significantly increased, while Prevotellaceae-Alloprevotella was significantly decreased. Among them, symbiotic bacteria such as Prevotellaceae-Alloprevotella could effectively prevent the intestinal colonization of Escherichia coli, Shigella and other pathogens;\(^{[35]}\) while Escherichia-Shigella is a typical intestinal pathogenic bacterium associated with diarrhea\(^{[36]}\), and could suppress the growth of normal flora and cause disorder of the flora. The increased diarrhea score in the model group showed that the animal model of intestinal flora disorder after 5-Fu chemotherapy was successfully prepared and the changes of Intestinal flora may be the key factor. Further studies showed that the severity of diarrhea in BPHW group was significantly lower than that in model group. The content of Bacteroides in BPHW group were effectively increased and the proportion of Proteobacteria were effectively reduced, especially the content of Escherichia-Shigella, which was significantly lower than that in model group and BLC group. However, Lactobacillus, which has obvious changes in the literature\(^{[37]}\), has no obvious improvement effect, suggesting that the elimination of pathogenic bacteria may be the first step for the Chinese medicine to anti-microecological damage which caused by chemotherapy. In addition, the imbalance of intestinal mucosal immunity induced by Th17/Treg is an important factor causing the disorder of flora by chemotherapy. In this study, we found that the expression of IL-6 and TGF-\(\beta\) increased significantly after 5-Fu intervention. The synergistic effect of the two factors induces ROR\(\gamma\)t differentiation and promotes the significant increase of Th17 effector molecule IL-17, TNF-\(\alpha\) and IL-1\(\beta\). Interestingly, unlike the antagonism of Th17 and Treg in chronic inflammatory bowel disease, we found that FOXP3 and IL-10 were also
significantly increased in the model group, which may be related to the transformation of Th17 and Treg mediated by IL-6, IL-23, ATRA and the response of acute intestinal stress. Further studies showed that the levels of TNF-α, IL-1β, IL-6 and TGF-β in BPHW group were both decreased. It was shown that BPHW inhibited IL-17 and RORγt expression and showed better immunomodulatory and anti-inflammatory effects.

In conclusion, BPHW decoction can inhibit the proliferation of intestinal pathogens rats undergoing chemotherapy and alleviate the symptoms of diarrhea. Evaluation of the underlying mechanisms revealed that BPHW may act by regulation Intestinal immunity and Treg/Th17 signaling pathway may be the potential mechanism. These data reveal that BPHW decoction is an effective approach for prevention and treatment of intestinal dysbiosis after chemotherapy. However, the traditional Chinese medicine compound is rather complicated, hence, the specific active components require further investigation.

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