Baogong decoction treats endometritis in mice by regulating uterine microbiota structure and metabolites

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Abstract
Endometritis is persistent inflammation caused by bacteria, which can lead to infertility. Although traditional Chinese medicine (TCM) has been used to treat endometritis, the underlying mechanism is still unclear. Here, Baogong Decoction (BGD), a TCM compound, was used to treat mouse endometritis induced by Escherichia coli (E. coli), and then 16S rRNA sequencing and non-targeted metabolomics were used to investigate the change of uterine microbiota and metabolomes in serum and uterine after BGD treatment. Finally, the therapeutic effect of potential metabolites for treating mouse endometritis screened by combined omics analyses was verified using pathological model. The results showed that BGD treatment could effectively treat endometritis associated with the increasing relative abundance of Firmicutes, Bacteroides, Lactobacillus and Lactococcus, and the decreasing relative abundance of Cupriavidus and Proteobacteria. 133 and 130 metabolites were found to be potential biomarkers in serum and uterine tissue respectively. In serum and tissues, dehydroepiandrosterone (DHEA) and catechol were significantly increased in the BGD treatment versus the inflammation group. Results of combined omics analyses demonstrated that DHEA was positively correlated with changes in microbiota. Results of pathological model demonstrated that DHEA could cure endometritis effectively associated with the decreasing infiltration of inflammatory cells and expression of inflammatory factors in the uterus. In summary, our results demonstrated that BGD could cure endometritis in mice by modulating the structure of the uterine microbiota and its metabolites, in which DHEA may be one of the main components of the therapeutic effect of BGD.
INTRODUCTION

Endometritis is a local inflammation mainly caused by *Escherichia coli* (*E. coli*), Mycobacterium pyogenes and Staphylococcus aureus (Piersanti et al., 2019). Endometritis can lead to many adverse health outcomes, and persistent inflammation can lead to infertility (Kitaya et al., 2018). A survey found that the prevalence of chronic endometritis was 2.8%–56.8% in infertile women and 9.3%–67.6% in women with recurrent miscarriage (Kimura et al., 2019). The incidence of endometritis after caesarean section is as high as 27%. Various antibiotic regimens such as gentamicin/clindamycin and aminglycoside combined with penicillin therapy are used to treat endometritis, but there are still some of treatment failures and allergic reactions (Mackeen et al., 2015). At present, antibiotics are the main drugs for the treatment of endometritis, but bacterial resistance also need attention (Li et al., 2015). The use of antibiotics can increase bacterial resistance, such as the increased resistance of *Bacteroides fragilis* to clindamycin (Aldridge & Sanders, 2002). *E. coli* is also increasingly resistant to antimicrobials (Careto et al., 2017). Antibiotics not only kill harmful microbiota, but also beneficial microbiota, leading to an imbalance in the body’s microbiota. The homeostasis of microbiota is very important for health (Neuman et al., 2018). Antibiotic resistance kills at least 700,000 people worldwide each year. Without treatment options to replace antibiotics, the World Health Organization predicts that the number could rise to 10 million by 2050 (Mancuso et al., 2021). Therefore, it is very important to reduce the use of antibiotics in diseases. It is imperative to find an alternative solution to antibiotic treatment.

Traditional Chinese medicine (TCM) has been used to treat endometritis with the advantages of high safety, few side effects and no residual antibiotics (Wei et al., 2017, Hu et al., 2020b, Lv et al., 2021). However, its mechanism needs to be elucidated. Therefore, it is particularly important to explore the underlying mechanism of TCM actions to improve treatment efficiency. It is thought that TCM treatment acts via two pathways. One is to achieve therapeutic effects by changing the composition of microorganisms, and the other is that TCM are metabolized into various small molecule compounds in the body and exert therapeutic effects through blood circulation (Su et al., 2016). Advances in metabonomic and microbiome technology offer new approaches to understand key switches in microbiota structure and metabolites before and after TCM treatment of diseases and to identify the mechanism of TCM actions (da Silveira et al., 2020).

Metabolomics provide the opportunity to find functional small molecules and assess pharmacological effects (Banoei et al., 2017). Previous studies showed that the efficacy of TCM involves blood metabolism. Metabolomic results showed that eight common metabolites were significantly reduced in models of pig myocardial infarction (Zhao et al., 2012). Metabolomic analysis of urine and plasma revealed that different pathways were involved in mouse pneumonia models established with different microorganisms (Lu et al., 2012). Baoyuan Decoction (BYD) could treat isoproterenol-induced myocardial hypertrophy in rats by interacting with intestinal microbiota. Metabolome analysis showed that the metabolites in rats treated with BYD for 4 weeks were basically the same as those in normal rats (Du et al., 2020).

In addition to metabolism, genital tract microbiota has an essential effect in the occurrence and development of endometritis (Osawa, 2021). A previous study showed that mice with endometritis had increased relative abundances of *Klebsiella*, *Lachnoclostridium_5* and *Citrobacter*, which may be responsible for endometritis (Dong et al., 2021). It is also showed that the relative abundance of *Lactobacillus* was reduced in the samples with endometritis (Lozano et al., 2021). Another study found that the *Lactobacillus buchneri* DSM 32407 helped improve the uterine environment (Peter et al., 2018). These findings suggest differences in microbial community structure in healthy and endometritis uterus. Changing the types or prevalence of microbiota may provide a new avenue to treat or prevent the occurrence of endometritis.

Baogong Decoction (BGD) is a TCM formula composed of Bupleurum, Gardenia, gentian, Licorice, Mutong, Phellodendron, Plantago, Poria and Red Peony. In the present study, in order to emphasize the mechanism of TCM in the treatment of endometritis, BGD was used to treat mouse endometritis induced by *E. coli*, and then analysis of blood and uterine metabolomes and microbiomes were used to reveal a potential mechanism of BGD actions.

EXPERIMENTAL PROCEDURES

Baogong Decoction

All TCM were purchased from the Changchun Chinese Medicine Market. BGD is a combination of Akebia quinata, Bupleurum, Cortex Phellodendri, Gardeniae fructus, gentian root, licorice root, Plantago asiatica L., Poria cocos and Radix Paeoniae Rubra. The medicinal materials were soaked in 2 L water for 30 min and boiled for 30 min to obtain the first treatment medicine, then add 1.5 L water and continue to boil for 30 min to obtain the second treatment medicine. The two medicines were mixed and filtered with gauze to obtain the final TCM used in the experiments.

Animals and experimental design

Ethical approval for the present study was obtained from the Ethical Committee of the Jilin Agricultural University, China.
BALB/c mice (150 females) aged 8–12 weeks were purchased from Liaoning Changsheng Biological Company. The mice were accommodated in individual cages, fed with standard diet at 24 ± 1°C and 60% humidity, and kept on a 12-h normal illumination cycle for 1 week before the experiments. Seven days after acclimation, mice were injected with pregnant mare serum gonadotropin (10 IU/mouse), and then with human chorionic gonadotropin (5 IU/mouse) the same time the next day. After 24 h, each 10 mice were randomly housed in a micro-isolated cage and subjected to experiments.

Culture Escherichia coli in Mueller-Hinton II cation-adjusted broth (BD, USA), then resuspend Escherichia coli in 1 ml of normal saline to a concentration of 1 × 10^{10} cfu/ml. Escherichia coli was isolated from cows with endometritis in our laboratory. The E. coli-induced endometritis mouse model was established in accordance with laboratory standards. Briefly, 25 μl of a mixed E. coli suspension was inoculated into the uterus of anaesthetized animals with a 19-mm soft needle (outer diameter 0.7 mm) to induce endometritis.

According to the experimental treatment, mice were randomly divided into control group, E. coli group and TCM group (1.15 g/ml TCM + E. coli). In the experiment of dehydroepiandrosterone (DHEA) treating mouse endometritis, the mice were randomly divided into control group, E. coli group, DHEA (1 mg/kg DHEA + E. coli) group and DHEA (2 mg/kg DHEA + E. coli) group. After 24 h of E. coli infection, BGD (300 μl) or DHEA (1 mg/kg or 2 mg/kg) was administered intragastrically at 8:00 am and 8:00 pm daily in the TCM group. The control group and E. coli group were given the same volume of saline. On the fifth day of treatment, all mice were placed in a cage and anaesthetized with chloral hydrate, and blood was collected and immediately centrifuged at 3500 g at 4°C for 20 min. Serum was then collected and stored at −80°C for metabolic analysis.

The uterine tissue was disected and weighed, and 150 μl of normal saline was injected into the uterus with a 19-mm soft needle for repeated washing to obtain the uterine rinsing solution for 16S rRNA analysis. The uterus was divided into two parts, one was stored at −80°C for metabolomic analysis and the other was stored in 10% neutral formalin buffer solution for histological analysis.

**Histopathological evaluation of uterine tissue**

The uterine tissue was placed in 10% formalin for 48 h, and then the tissue was embedded in paraffin and processed into 4-μm sections. Pathological evaluation under light microscope after haematoxylin and eosin staining.

**Real-time quantitative PCR (RT-qPCR)**

Total RNA from the uterine tissue was extracted using TRIzol. The steps for RNA reverse transcription and RT-qPCR were the same as before (Ding et al., 2020). PrimeScript™ RT reagent Kit and SYBR® Premix Ex Taq™ II for reverse transcription and RT-qPCR processes respectively. RT-qPCR was performed in Agilent Stratagene Mx3005P qPCR system (Agilent Technologies, Inc.). These RT-qPCR assays were run in triplicate for each sample, and the results were normalized to the expression of β-actin. The relative gene expression was analysed by 2^{−ΔΔCt} comparison method. Primer sequences is provided in Table 1. The RT-qPCR reaction program is shown in Table 2.

**ELISA**

The uterine tissues were harvested and homogenized with PBS on ice. The samples were collected and centrifuged at 2000 g for 20 min at 4°C. And the supernatants were used to assay levels of pro-inflammatory cytokines interleukin-1 (IL-1β), interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α) using ELISA kits according to the manufacturer’s instructions (Meimian biology). Absorbance values were read at 450 nm.

**DNA extraction and 16S rRNA sequencing**

The rapid DNA SPIN extraction kit was used to extract total DNA from uterine samples. The PCR products

| Gene | Sequence of primers | Tm (°C) | Concentration (μM) |
|------|---------------------|---------|-------------------|
| IL-6 | F: CCACCTTCAAGATGGAGGCTTA  
R: CCAGTTTGAGCATCCATATTTC | 60 | 10 |
| IL-1β | F: TCGGAATTCCAGGACAGATGAGAC  
R: GGAGTAGAACAGCAGGGTATA | 60 | 10 |
| TNF-α | F: TATGGCACGACCCCTGA  
R: GAGATGAGAACAGCAGGGTATA | 60 | 10 |
| β-Actin | F: CTCTTATGGACGATGAGAC  
R: TTGATCTTATGGACGATGAGAC | 60 | 10 |

| Tm (°C) | Time (s) | Cycles |
|---------|----------|--------|
| 95      | 30       | 1      |
| 95      | 5        | 40     |
| 60      | 34       | 1      |
| 60      | 60       | 1      |
| 95      | 15       | 1      |

**Histopathological evaluation of uterine tissue**

The uterine tissue was placed in 10% formalin for 48 h, and then the tissue was embedded in paraffin and processed into 4-μm sections. Pathological evaluation under light microscope after haematoxylin and eosin staining.
RESULTS

Establishment of mouse endometritis model

We used the *E. coli* perfusion method to establish a mouse endometritis model. After 24 h of perfusion with different concentrations of *E. coli*, 10⁷ and 10¹⁰ cfu/ml *E. coli* significantly up-regulated the expression of IL-1β, IL-6 and TNF-α (Figure 1A,B). After testing the effective duration of inflammation caused by 10¹⁰ cfu/ml *E. coli*, it was found that within 7 days, *E. coli* had a continuous inflammation treatment effect (Figure 1C,D).

BGD treatment of *Escherichia coli*-induced endometritis in mouse

Baogong Decoction was used to treat mice endometritis induced by *E. coli* and histopathological changes in the mice uterus were examined. As shown in Figure 2A, uterine tissue of the *E. coli* group showed hyperaemia, oedema and degeneration, and haematoxylin and eosin staining showed inflammatory cell infiltration. In addition, *E. coli* significantly up-regulated the levels of IL-1β, IL-6 and TNF-α (Figure 2B,C). TCM treatment reduced uterine tissue congestion and some inflammatory cell infiltration, and significantly reduced the levels of cellular inflammatory factors.

BGD regulates the microbiota in mice with endometritis

In this study, Life ION S5 sequencing of the V3-V4 regions of 16S rRNA was performed to explore the influence of TCM on uterine microbiota. A Venn diagram showed that there were 1529 unique OTUs in the *E. coli*-treated group, 3460 unique OTUs in the *E. Coli* TCM-treated group, and 541 OTUs common to both groups (Figure 3A). The study revealed that, compared with the *E. coli* treatment, the composition of microbiota became relatively complex after TCM treatment. Weighted uniFrac-based PCoA to evaluate the overall microbiota structure of each group (Figure 3B). There appeared to be distinct clusters between the *E. coli*-treated and *E. coli*-TCM-treated groups. The result showed that TCM altered the overall composition of uterine microbiota in mice with endometritis. The alpha diversity of microbiota after TCM treatment was significantly higher than that of the *E. coli* group, and different indices, including chaot, Shannon, and Faith-pd, had the same trend (Figure 3C). To determine which bacteria was impacted by TCM treatment, high-dimensional class comparisons were performed using LEfSe, which revealed significant differences in the major bacterial communities (Figure 3D). Proteobacteria, *Cupriavidus*...
and Lysobacter were the key bacterial types for *E. coli* to induce endometritis. Actinobacteria, Bacteroidetes, Firmicutes and *Ralstonia* showed were significantly enriched in the TCM group, changes which may be related to effective treatment of endometritis. Based on bacterial OTU abundance, a comparative heat map was used to analyse the uterine microbiota of the two groups, and the findings were consistent with the results of LEfSe analysis (Figure 3G–H). To further identify the differences in species composition between the two samples, taxonomy was used to analyse species composition. As shown in Figure 3E, compared

**Figure 1** Effects of *Escherichia coli* (*E. coli*) on the expression of uterine inflammation factors interleukin-1β (IL-1β), IL-6 and tumour necrosis factor-α (TNF-α) in mice. (A, B) Effects of different concentrations of *E. coli* on the expression of IL-1β, IL-6 and TNF-α mRNA and protein, *n* = 12. (C, D) Effects of 10^10 cfu/mL *E. coli* on the expression of IL-1β, IL-6 and TNF-α mRNA and protein within 7 days, *n* = 10. *p < 0.05, **p < 0.01, ***p < 0.001 compared with the control group.
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with the E. coli group, Proteobacteria decreased from 94.48% to 67.84%, Firmicutes increased from 2.62% to 13.30%, Bacteroidetes increased from 1.62% to 9.44%, Actinobacteria increased from 0.56% to 4.01% and Spirochaetes increased from 0.22% to 2.27% after TCM treatment. At the genus level (Figure 3F), compared with the E. coli group, Ralstonia increased from 11.78% to 45.58%, Pelomonas increased from 1.59% to 7.07%, Bacteroides increased from 0.29% to 2.56%, Cupriavidus decreased from 44.90% to 6.12% and Lysobacter decreased from 11.75 to 0.09% after TCM treatment. Collectively, TCM treatment significantly altered the diversity and constitution of the microbiota.

BGD regulates serum and uterine tissue metabolism in mice with endometritis

OPLS-DA was used to analyse the metabolic changes in serum and uterus of mice in the E. coli group and TCM group (Figure 4A,B). It was observed that the two groups were completely separated, indicating that the sequencing results were stable and effective. Candidate metabolites were screened by OPLS-DA model with VIP > 1 and p < 0.05, where metabolites with an error of less than 5 ppm between the experimental and extracted mass values were screened as potential biomarkers. In conclusion, 133 and 130 metabolites were found to be potential biomarkers in serum and uterine tissue respectively. In serum, there were 74 upregulated metabolites and 59 downregulated metabolites in the TCM group compared to the E. coli group. In uterine tissue, there were 79 upregulated metabolites and 51 downregulated metabolites in the TCM group compared to the E. coli group. In serum and tissues, DHEA and catechol were significantly increased in the TCM treatment compared with the E. coli group, and they may be potential products for the treatment of endometritis in mice (Figure 4C, serum; D, uterus). We identified and analysed the differential metabolites between the two groups, and screened out metabolic pathways with an impact value >0.01 through KEGG analysis. We found that the serum and uterus shared three metabolic pathways, namely glyoxylate and dicarboxylate metabolism, arginine and proline metabolism, and histidine metabolism (Figure 4E, serum; F, uterus). These may represent potential metabolic pathways targeted by TCM to treat endometritis in mice.

The relationship between uterine microbiome and metabolome

To further investigate the mechanism of TCM in treating endometritis in mice, we visualized the correlation between the uterine microbiota and metabolites, and performed Spearman correlation analysis on the relative abundance of different microbial species and metabolites. Among the 30 metabolites shown in the resulting heat map, 28 metabolites had at least one significant correlation with microorganisms. Seventeen metabolites were positively correlated and nine
DING et al. metabolites were negatively correlated with microorganisms. Metabolite changes induced by TCM treatment were associated with changes in microbiota. The current study found that DHEA level was significantly increased after TCM treatment, and DHEA had a positive correlation with changes to most of the microbiota (mainly *Bacteroides*). The results indicated that DHEA plays a role in the TCM treatment process of mouse endometritis induced by *E. coli*. Therefore, DHEA was used for treatment experiments in mice (Figure 5).

**Effects of DHEA on mouse endometritis**

The therapeutic effect of DHEA was examined in the mice endometritis model. As shown in Figure 6, DHEA reduces *E. coli*-induced uterine hypertension and oedema, and decreases inflammatory cell infiltration. In addition, 2 mg/kg DHEA significantly decreased the expression of IL-1β, IL-6 and TNF-α mRNA and protein ($p < 0.05$). Our results showed that DHEA treated mouse endometritis, although various inflammation indicators did not return to normal levels.

**DISCUSSION**

Traditional Chinese medicine has applications in a variety of diseases and its mechanism of action is complex (Genis et al., 2017; Lin et al., 2019). Previous studies have shown the efficacy of TCM in treating diseases (Wang et al., 2015, Wang et al., 2019, Hu, et al., 2020). In particular, TCM could regulate the structure and metabolism of microbiota, and conversely, microbiota can also metabolize TCM compounds (Feng et al., 2019). The present study found that TCM could treat mice...
endometritis caused by \textit{E. coli} effectively, and reduce uterine tissue swelling, inflammatory infiltration and the levels of IL-6, IL-1\(\beta\) and TNF-\(\alpha\). At present, IL-6, IL-1\(\beta\) and TNF-\(\alpha\) are the commonly used indicators to detect endometritis, and these three inflammatory factors can be used as biomarkers for chronic endometritis (Tortorella et al., 2014). Investigation of microbiome and metabolome showed that the effect of TCM on
Endometritis may involve small-molecule metabolites acting on uterine tissue through blood circulation and causing changes in uterine microbiota structure.

As reported in other studies, the composition and function of the reproductive tract microbiome play an important role in endometritis (Bicalho et al., 2017) and bacteria is key player in endometritis (Wang et al., 2020). Evaluation of the intrauterine microbiota by 16S rRNA sequencing found that the uterus is rich in microbiota and bacteria is the main cause of the differences in intrauterine microbiology (Miranda-CasoLuengo et al., 2019). The current study found that TCM treatment increased the abundance of Firmicutes and Bacteroides, and reduced the relative abundance of Cupriavidus and Proteobacteria. In addition, the results showed that Proteobacteria, Cupriavidus and Lysobacter were the key bacteria affected by E. coli-induced endometritis, and the abundance of Actinobacteria, Bacteroidetes, Firmicutes and Ralstonia were higher in the TCM-treated group. Past work showed that Firmicutes were more abundant in the uterus of healthy mammals, and at the phylum level, Proteobacteria was more abundant in animals with endometritis and less abundant in healthy animals.

**FIGURE 5** The interaction between the uterine microbiota and metabolome. *p < 0.05 and **p < 0.01 indicate significant differences between the microbiota and metabolites. Blue represents positive correlations, red represents negative correlations.
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(Wang et al., 2017), which is consistent with our results. In mice with colitis, epigallocatechin-3-gallate or faecal microbiota transplantation could reduce intestinal inflammation and maintain colonic barrier integrity by modulating gut microbiota (Wu et al., 2021). Faecal microbiota transplantation attenuated endometritis in mice, and the diversity of faecal microbiota, including the abundance of Bacteroides and Firmicutes, was significantly increased after transplantation (Hu, et al., 2020a). A significant decrease in diversity of uterine microbiota was reported in the endometritis group compared to the non-endometritis group (Chen et al., 2021; Pascottini et al., 2020), which is consistent with our results.

In addition to changing the structure of the microbiota, TCM could be metabolized into small molecular compounds, which reach target tissues via blood circulation to achieve therapeutic effects (Gong et al., 2020). Previous findings indicated that Ermiao fang alleviated endometritis in rats, and of the 24 compounds in Ermiao fang identified by ultra-high performance liquid chromatography, eight were absorbed into the circulation after oral administration to potentially reduce inflammation (Zhang et al., 2020). TCM could produce a variety of metabolites, such as vitamins and short-chain fatty acids (Nicholson et al., 2012). It could also regulate homeostasis and disease development through interaction with the host (den Besten et al., 2013). The present study used metabolic analysis of the serum and uterus of mice in the E. coli group and TCM group. This analysis identified 133 potential biomarkers in the serum, of which 74 were up-regulated and 130 potential biomarkers in the uterus, of which 79 were up-regulated, including taurine, ursodeoxycholic acid, mannitol and DHEA. Taurine and ursodeoxycholic acid were significantly increased in serum and uterus, respectively, in the TCM-treated group. It has been reported that taurine can reduce the inflammatory response in type 2 diabetes patients and rats with cervicitis (Maleki et al., 2020; Zhang et al., 2018). Ursodeoxycholic acid was used to treat a variety of diseases, including inflammatory bowel disease and mucositis (Keely et al., 2019; Kim et al., 2018), and affects the intestinal microbiota (Pearson et al., 2019). Both mannitol and DHEA were significantly increased in serum and uterine tissue after TCM treatment. Mannitol is a polyol with anti-inflammatory effects (Cavone et al., 2012), and clinically mannitol can treat some diseases such as migraine and ischaemia–reperfusion (De Simone et al., 2017; Goksin et al., 2011). DHEA levels were highest in the uterus after TCM treatment. DHEA is a hormonal metabolite that alters immune responses (Stárka et al., 2015). In the elderly, DHEA exerts an immunomodulatory effect, increasing the number of monocytes and T cells. It relieves inflammatory bowel disease in most of the patients. In women, DHEA improves fertility and age-related vaginal atrophy. DHEA is a useful drug for human disease (Rutkowski et al., 2014). TCM treatment of endometritis in mice

FIGURE 6 Effects of dehydroepiandrosterone (DHEA) on mouse endometritis. Mouse uterine tissues were collected from control, E. coli and E. coli + DHEA (1 and 2 mg/kg) groups. (A) Morphological changes to uterine tissue (left), and haematoxylin and eosin-stained sections of uterine tissue (right, 100x), n = 8. Uterine expression of interleukin-1β (IL-1β), IL-6 and tumour necrosis factor-α (TNF-α) mRNA (B) and protein (C). **p < 0.05 and ***p < 0.001 compared with the control group, n = 10. #p < 0.05 and ##p < 0.01 compared with the E. coli group.
can increase the levels of various metabolites, thereby relieving endometritis in mice. These metabolites, DHEA has the highest expression in the uterus after TCM treatment and plays an important role in women's health. Therefore, DHEA is our focus of attention. In addition, TCM treatment of endometritis in mice could also reduce some harmful metabolites, such as 5-Hydroxyindoleacetic acid. Studies have reported that 5-Hydroxyindoleacetic acid was significantly positively correlated with inflammation in pelvic inflammatory disease, and TCM treatment could reduce the level of 5-Hydroxyindoleacetic acid (Zhang et al., 2022). In a rat disease model, 5-Hydroxyindoleacetic acid was significantly positively correlated with acute myocardial infarction, and TCM Shexiang Baoxin Pill could reduce the level of 5-Hydroxyindoleacetic acid and protect the cardiovascular system (Wu et al., 2020). The biomarkers jointly participate in three metabolic pathways, glyoxylic acid and dicarboxylic acid metabolism, arginine and proline metabolism, and histidine metabolism. It has been shown that TCM affects host metabolism (Li et al., 2019). In addition, our results showed that metabolites in the TCM treatment group were positively correlated with specific microbial groups, mainly in the Bacteroides. Overall, the Bacteroides might be a key factor that changes the structure of microbiota and affects host metabolism. The potential relationship between the function of uterine microbiota and the metabolome provide new insights into the functional role of TCM and the production of small molecular metabolites in the treatment of endometritis.

The current study screened the common metabolites of serum and uterine metabolism, and from the combined analysis of the uterine microbiome and metabolome, the metabolite DHEA was found to be positively correlated with most bacteria after BDG treatment of mouse endometritis. Moreover, our studies indicated that DHEA administration improved the pathological changes in uterine tissue and reduced the occurrence of inflammation in E. coli-induced endometritis, although uterine changes had not returned to normal levels. We predict that TCM can treat diseases through metabolism. DHEA is a key metabolite of cholesterol and affects the inflammatory response of animals (Zhao et al., 2020), and has been shown to affect immune function (Yang et al., 2016). DHEA appears to treat E. coli-induced enteritis in mice through reducing the expression of pro-inflammatory factors. DHEA has been shown to improve immune cell function by promoting the production of IL-4 and IL-10 (Zhao et al., 2020), and reduce inflammation by inhibiting IL-6 and TNF-α, and lower DHEA levels are associated with a variety of human diseases (Traish et al., 2011). Previous studies also found that DHEA blocks the production of pro-inflammatory factors and their mediators by inhibiting the NF-KB pathway (Jeon et al., 2015). The anti-inflammatory and antibacterial effects of DHEA are also attributed to the inhibition of lipopolysaccharide-mediated production of pro-inflammatory factors (Alexaki et al., 2018). Oral DHEA increases ovarian reserve and pregnancy rates in women with poor ovarian response (Ozcil, 2020). It has been reported that the combination therapy of TCM formula Ziyin Jianghuo Ningxin Decoction and DHEA has good effects in controlling menopausal symptoms and restoring hormone levels in women (Lin et al., 2018). At present, the treatment of diseases by DHEA in TCM is mostly carried out in mouse disease models. A study reported that Bu–Shen–Ning–Xin decoction inhibited postmenopausal osteoporosis by increasing DHEA (Gui et al., 2015). In addition, DHEA was able to alleviate colitis in mice and reduce the abundance of Pseudomonas (Cao et al., 2020). This indicates that in different disease models, DHEA may reduce inflammation by regulating different microbiota. TCM has been widely used in the treatment of diseases, and research has also found that Xiaoyaosan can increase the abundance of Bacteroides to treat colorectal cancer (Zhao et al., 2021). Compound Agrimony Enteritis Capsules can increase the relative abundance of probiotics in Bacteroides and inhibit IL-6 production (Li et al., 2021). In addition, the TCM Gui-fu-di-huang-wan can increase the abundance of Bacteroides in the body and regulate the microbiota structure and metabolite levels (Jing et al., 2021). Numerous studies have demonstrated the effectiveness of TCM and DHEA in the treatment of human diseases, and that TCM can modulate the abundance of microbiota, including Bacteroides. The above studies show that DHEA has anti-inflammatory ability and support our current findings. Studies have showed that TCM relies on intestinal microbiota to play a role in curing diseases (Du et al., 2020). However, because of the lack of knowledge about the relationship between intestinal microbiota and reproductive tract microbiota, and their contribution to endometritis, these functional interrelationships need to be evaluated in future studies. The current data suggest that screening small-molecule compounds affected by TCM-induced metabolic changes may serve as new drug candidates for the treatment of the disease and may also increase our understanding of TCM for endometritis. The effectiveness and benefits of TCM replacement therapy should be further validated by comparison with antibiotics.

CONCLUSION

In summary, our results showed that BGD could treat mice endometritis through the host (serum) metabolism and blood circulation acting on the uterus, in particular, changing the structure and metabolism of uterine microbiota. Our results also showed that BGD may treat
endometritis at least partly via its metabolites (DHEA), and the abundance of the *Bacteroides* was positively correlated with DHEA.

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**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

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