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RESEARCH ARTICLE

Efficacy of Bushenjianpi prescription on autoimmune premature ovarian failure in mice

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Abstract

OBJECTIVE: To assess the efficacy of Bushenjianpi prescription (BSJPP), a formula from Traditional Chinese Medicine, on a mouse model of autoimmune premature ovarian failure (POF) induced by mouse zona pellucida (ZP3) and to investigate the mechanisms underlying the action.

METHODS: After randomization, POF was induced in the model mice by immunization with ZP3. One week later, mice received low (8.1 mg/kg), moderate (16.2 mg/kg) and high (32.4 mg/kg) doses of BSJPP by gastrogavage once daily for 90 days. Premarin (0.03 mg/kg) served as the positive group. Serum samples were collected 1 week after the last dose and stored at − 20 ℃ for analysis. After cervical dislocation, the uterus and ovaries were collected aseptically for evaluation by histological assessment, scanning electron microscopy, immunohistochemical staining, and Western blot and reverse transcription-polymerase chain reaction analyses.

RESULTS: Serum E2 levels in POF model mice were decreased, whereas follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels were dramatically increased. Serum levels of E2, LH and FSH were reduced in POF model mice treated with BSJPP (moderate and high doses) and premarin. Anti-bone morphogenetic protein 15 (BMP-15) and connexin 43 (Cx43) were repressed in autoimmune POF model mice, whereas high expression was observed in control mice and those treated with BSJPP (moderate and high doses) and premarin.

CONCLUSION: BSJPP is effective in treating ZP3-induced POF in mice and the increase in the expression of BMP-15 and Cx43 may be implicated in the mechanism underpinning the action.

Keywords: Chinese medical formula; Primary ovarian Insufficiency; Zona pellucida; Follicle-stimulating hormone; Luteinizing hormone; Connexin 43

INTRODUCTION

Premature ovarian failure (POF) is a heterogeneous syndrome with several causative factors.[1] Experimental and clinical data implicate autoimmunity as the pathogenic mechanism in 30% of POF.[2] The lack of a highly sensitive and specific test has precluded accurate estimation of the prevalence of autoimmune POF. Studies show that autoimmunity is responsible for approximately 4% to 30% of POF cases.[3] Lack of ovarian steroid synthesis has serious implications for women's health, with short-term symptoms and consequences similar to those observed in spontaneous menopause. From a longer-term perspective, POF reduces the chance of normal conception, leads to urogenital atrophy, interferes with sexual functioning, and decreases bone mineral density. These symptoms are not only associated with psychological distress, but can also lead to neurodegenerative diseases.[4]

Women suffering from POF are usually treated with hormone replacement therapy (HRT), which suppresses menopausal symptoms when administered with the combined oral contraceptive pill (COCP). However, no therapy is designed to achieve physiological replacement of estrogen and progesterone, an approach which is superior to standard hormone replacement.[5] Furthermore, clinical studies have indicated that long-term application of HRT significantly increases the risk of venous thrombo-embolism, stroke and gallbladder disease; thus, only short-term application of HRT appears to be safe for women with POF.[6]

Stem cell transplantation is considered to be an ideal potential treatment for POF. The effects of bone marrow transplantation on female reproductive function have been clarified in studies using a preclinical mouse model of chemotherapy-induced POF.[7] However, the lack of clarity regarding the exact mechanism underlying the effects of stem cell transplantation has impeded follow-up research.

Recent research has indicated the efficacy of Traditional Chinese Medicine (TCM) in the management of POF and its secondary diseases.[8,9] Nevertheless, the mechanism underlying the action remains unknown.

Bushenjianpi prescription (BSJPP) is a formula from TCM. BSJPP is derived from the formula used to prepare Shuangbu Decoction,[10] which was originally documented in medical literature "Wenbing Tiaobian" authored by Tang Wu in the Ming dynasty. BSJPP can manipulate immune responses and affect endocrine system involving hypothalamic-pituitary-gonadal axis, and have been used to manage climacteric syndrome for several years.[11,12]

Based on previous clinical studies and the various pharmacological properties of BSJPP, our study was designed to establish the association between BSJPP and POF by evaluating the efficacy of BSJPP and investigating the mechanism whereby BSJPP regulates relevant gene expression and hormone secretion to protect against POF.

MATERIALS AND METHODS

Experimental drugs

BSJPP, which is composed of prepared Dihuang (*Radix Rehmanniae*, 10 g), Lianzi (*Semen Nelumbinis*, 10 g), Shanyao (*Rhizoma Dioscoreae Oppositae*, 10 g), Tusizi (*Semen Cuscutae*, 10 g), Bajitian (*Radix Morindae Officinalis*, 10 g), Fupenzi (*Fructus Rubi Chingii*, 10 g), Huangjing (*Rhizoma Polygonati Sibirici*, 10 g), Shihu (*Herba Dendrobii Nobilis*, 10 g), Juye (*Folium Citri Reticulatae*, 10 g), Xiangfu (*Rhizoma Cyperi*, 10 g) and Gancao (*Radix Glycyrrhizae*, 3 g) was purchased from and identified by the Tongrentang Pharmaceutical Co., Ltd., (Beijing, China). The quality of these herbs was strictly controlled and processed according to the Chinese Pharmacopoeia (2010). The chemical compositions of all medicinal plants used in BSJPP have been analyzed.[12] Premarin (0.625 mg per tablet; Wyeth Pharmaceuticals: product lot No. H20100586) was purchased from the Fu Guan Tang Pharmacy (Guangzhou, China).

Reagents

Complete Freund's adjuvant (CFA; product lot No. F5881) and Incomplete Freund's incomplete adjuvant (IFA; product lot No. F5506) were purchased from Sigma (St. Louis, MO, USA). Mouse zona pellucida (ZP3) were produced by the Chinese Peptide Company (Hangzhou, China). Anti-connexin 43 (Cx43) antibody (product lot No. BA1727) and anti-bone morphogenetic protein 15 (BMP-15) antibody (product lot No. BA2018) were purchased from Boster Biological Engineering (Wuhan, China). Mouse E2, (product lot No. elisa2464) and rabbit anti-goat IgG were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, 95060, USA). Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) enzyme-linked immunosorbent assay (ELISA) kits (product lot No. ZA-0264 and ZA-0344, respectively) were purchased from ZSGB-Biotechnology (Beijing, China).

Instruments

The following instruments were used in this study: Phase contrast microscope (BX51, Olympus, Tokyo, Japan), low-speed desktop centrifuge (Eppendorf AG, Hanmburg, Germay; model, 5804); Vortex (IKA, Staufen, Germay, model MS3), water meter (Millipore, Boston, America; model D24UV), microplate reader (Bio-Rad, Hercules, CA, America; model 680). UV spectrophotometer (Shimadzu, Kyoto, Japan; model UV-2450).

Medicinal preparation

The medicinal preparation was prepared as previously described.[13] Briefly, BSJPP was steeped in distilled water (1∶8) for 20 min, decocted for 30 min, and filtered through eight layers of gauze. The decocted gruffs were again decocted in water (1∶6) for 30 min, and filtered