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Research Progress on the Mechanisms of Zuo Gui Wan for Treating Diminished Ovarian Reserve

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Abstract: ***Zuogui Pill is derived from Jing Yue Quan Shu (Jingyue's Complete Works). It is mainly used for the deficiency of kidney Yin and its function is to nourishing the kidney and filling the essence. It has been used clinically for more than 400 years and is now commonly used in the treatment of osteoporosis, knee osteoarthritis and other diseases. In recent years, more and more studies have shown that Zuogui pill has a certain effect in the treatment of Diminished ovarian reserve (DOR). This article summarizes the mechanism of Zuogui pill in the treatment of DOR from the aspects of metabolomics evidence, network pharmacological target prediction, regulation of sex hormone levels, promotion of ovarian germ stem cell proliferation, improvement of oxidative stress, and promotion of ovarian angiogenesis. From the molecular mechanism, it is revealed that Zuogui pill has the effect of treating DOR. This study systematically reviews the basic research progress of Zuogui pill in the treatment of diminished ovarian reserve (DOR), points out the limitations of the existing experimental system and puts forward optimization strategies, so as to provide theoretical support for the in-depth mechanism exploration, clinical transformation and new drug research and development of DOR.***

Keywords: Zuogui Pill, Decreased Ovarian Reserve, Mechanism of Action.

1. Introduction

Diminished ovarian reserve (DOR) refers to a decrease in the ability of a woman's ovary to produce eggs, including a decrease in egg quality and/or quantity [1]. The clinical manifestations are irregular menstruation, decreased fertility, and may be accompanied by perimenopausal symptoms such as sweating and hot flashes. There is no unified diagnostic criteria for DOR in clinical practice, and the incidence of DOR is about 10%-35% [2]. Without timely intervention, it may further cause amenorrhea, female infertility, or even premature ovarian failure, which will have a serious impact on women's reproductive health and fertility. For the treatment of DOR, western medicine mainly adopts hormone replacement therapy and ovulation induction measures for women with pregnancy preparation requirements [3]. Although the clinical efficacy is satisfactory, there are still some problems such as more adverse reactions, long medication cycle and recurrence after drug withdrawal. There is no disease name and diagnosis of DOR in traditional Chinese medicine (TCM), but according to the symptoms of DOR such as irregular menstruation, amenorrhea and decreased fertility, it is consistent with the descriptions of "pre-menstrual period", "less menstrual period", "dry menstrual period" and "infertility" in TCM classics. The long development process of TCM has certain treatment research and treatment experience for this disease. In the clinical treatment of DOR, traditional Chinese medicine (TCM), acupuncture and ear acupoint bean-pressing are commonly used. The combination of various therapies will become the main trend in the future.

Zuo GUI Wan is derived from Jing Yue Quan Shu (Jingyue's Complete Works) written by Zhang Jingyue. In volume 51, the new prescription of eight array of tonic array is often used to treat the syndrome of kidney-yin deficiency caused by the deficiency of true Yin, such as soreness and weakness of waist and knee, tinnitus and night sweat, fatigue and dry mouth, etc. It has the effect of treating the deficiency of true Yin and kidney water. Zuogui Pill is made up of eight herbs: ripe ground, yam, cornus officinalis, deer gum, turtle gum, lycium barbarum, cow's knee and dodder seed. Some studies have shown that Zuogui pill can relieve the clinical symptoms of DOR patients with kidney Yin deficiency, such as waist and knee soreness, hot flashes and sweating, menstrual cycle, low menstrual volume, menstrual color and other symptoms [4]. In this paper, the relevant literature in recent years was searched and sorted out, and the mechanism of action of Zuogui pill in the treatment of DOR was summarized, aiming to provide theoretical basis and scientific ideas for the in-depth study of Zuogui pill and the development of new drugs for DOR.

2. Metabonomic Evidence and Network Pharmacological Target Prediction

Metabonomics is the qualitative and quantitative analysis of endogenous small molecules through high-throughput technology and bioinformatics methods. This technique can reveal the differences in body metabolism of complex chemical components of traditional Chinese medicine in disease states such as DOR. Therefore, from the perspective of network metabolism, this study explains the mechanism of Zuogui pill in the treatment of DOR with kidney Yin deficiency syndrome and identifies potential biomarkers, which provides molecular evidence for elucidating the material basis of its drug effect. Based on serum metabolomics, Cao Ying et al. [5] studied Zuo GUI pill in the treatment of DOR based on syndrome differentiation. The results showed that Zuo GUI pill intervention could regulate 26 differential metabolites (such as L-carnitine, acetyl-coa, coenzyme A, coenzyme Q10) in DOR patients with kidney Yin deficiency, involving 12 related metabolic pathways, and playing A positive therapeutic role in DOR patients with

kidney Yin deficiency.

Network pharmacology is based on the interaction between diseases, genes, targets and drugs. By constructing networks (such as the "key TCM combination-target-disease" network), it comprehensively studies the influence of drugs on diseases from the system level, which is in line with the holistic view of "multi-component, multi-target and systematic regulation" of traditional Chinese medicine [6]. Cao Ying [7] screened 143 active ingredients and corresponding 448 potential targets of Zuo GUI pill based on network pharmacological analysis. Combined with 113 genes related to reduced ovarian reserve (DOR) and kidney Yin deficiency syndrome, 39 key targets (such as TP53, VEGFA, ALB) of Zuo GUI pill in the treatment of DOR syndrome were finally identified. Zuogui pill may act synergistically on the above targets through multiple components to regulate molecular functions including reproductive system development, hormone response (especially steroid hormones), cell membrane and organelle outer membrane structure, steroid/lipid binding, protein kinase/phosphotransferase activity, atpase binding and so on. It is involved in PI3K-Akt, HIF-1, estrogen, JAK-STAT, MAPK signaling pathways and cell senescence. Molecular docking verification showed that the binding energies of the core active components to the key targets were all negative, suggesting the existence of stable binding. In conclusion, this study preliminarily reveals the potential multi-target and multi-pathway mechanism of Zuogui pill in the treatment of DOR syndrome based on syndrome differentiation.

3. Regulates Sex Hormone Levels

In current clinical practice, the assessment of ovarian reserve function is mainly based on age, combined with serum Anti-Mullerian hormone (AMH), Antral follicle count (Antral follicle count, antral follicle count), and ovarian reserve function. AFC) and basal follicle stimulating hormone (FSH) levels were comprehensively analyzed [1]. AMH, a member of Transforming growth factors-β (TGF-β) superfamily, is secreted by ovarian Granular cells (GC) and is the only endogenous factor known to specifically inhibit the growth of primordial follicles [8]. AMH expression is strictly restricted to the follicular growth phase before FSH-dependent selection [9] and is not expressed in luteal phase follicles and atretic follicles. Therefore, serum AMH level in women is highly consistent with ovarian reserve, which can reflect ovarian reserve function and is an important marker for evaluating this index. Antral follicle count (AFC) refers to the total number of basal follicles in both ovaries assessed by transvaginal ultrasound during the menstrual phase. Clinical studies have shown that AFC is significantly correlated with female reproductive age [10]. Follicle stimulating hormone (FSH) is a gonadotropin secreted by eosinophils in the anterior pituitary gland, which is routinely used in the ovulation induction protocol of assisted reproductive technology (ART) [11]. Basal serum FSH concentration in women is a marker of ovarian reserve function, and its increase indicates decreased ovarian reserve function. The follicle stimulating hormone receptor (FSHR) is specifically expressed in granulosa cells and triggers a signaling cascade upon binding to its ligand FSH. This process starts in the late preantral follicular phase, promotes follicle recruitment, stimulates granulosa cell proliferation, induces aromatase synthesis, and finally catalyzes the conversion of androgen to estrogen [12]. FSH and luteinizing hormone (LH) stimulate target organs to secrete estradiol (E2), androgen and other sex hormones, thereby regulating female reproductive function [13]. Some studies have shown that [14] when ovarian function is damaged and ovarian function is decreased, the level of estradiol in animals will be reduced.

A series of studies have shown that Zuogui pill can improve the serum AMH level of rats with chemotherapy-induced ovarian function decline [15]. Decreased serum FSH and LH concentrations, increased AMH, E2 and FSHR concentrations, increased the number of primary follicles and growing follicles, and decreased atretic follicles in the cyclophosphamide (CTX) -induced DOR mouse model [16]. All these studies suggest that Zuogui pill can improve ovarian reserve function by regulating sex hormone levels in women.

4. The Proliferation of Ovarian Germ Stem Cells

oogonial stem cells (OSCs) are a subset of stem cells newly identified in the past decade, which exist in the postnatal ovary. Its finding challenges the "fixed follicle pool" theory, and indicates that OSCs are located in the ovarian microenvironment (stem cell nest) and can differentiate into oocytes under the regulation of cell-factor network, thus supplemting the ovarian reserve [17]. In 2004, Johnson et al. [18] identified the germ cell-specific marker mouse vasa homologue (MVH) in the mouse ovarian epithelium, and Mvh is a recognized marker of mammalian germ cells. Octamer binding transcription factor 4 (Oct4) is a specific transcription factor for ovarian female germ stem cells (FGSCs) [19]. At present, Mvh and Oct4 are commonly used to identify ovarian germ stem cells. zonapellucida (ZP) is composed of glycoproteins ZP1, ZP2, ZP3 and ZP4. The synthesis of ZP starts at the stage of primary follicle, and its thickness gradually increases with the development of follicle [20], which plays an auxiliary role in the identification and evaluation of ovarian germ stem cells. Studies have confirmed that Notch signaling pathway can maintain the "stemness" of stem cells [21] and also mediate stem cell differentiation [22]. In mammals, Pan Zezheng [23] found that Notch signaling pathway regulates the function of mouse ovarian reproductive stem cells and the number of primordial follicles. Liang Xia et al. [24] found that the Notch pathway is highly expressed in ovarian germ stem cells, and the inhibition of this pathway leads to the decrease of their proliferation activity. Notch pathway regulates cell differentiation, proliferation and apoptosis. The expression levels of its downstream key molecules Notch1, Hes1 and Hes5 can indicate the intensity of pathway activity.

Ye Jinfei [15] found that high-dose Zuogui pill treated rats with decreased ovarian function could significantly up-regulate the expression of ovarian germ stem cell markers Mvh, Oct4 and related genes ZP1 and ZP3, suggesting that Zuogui pill could improve ovarian reserve by increasing the number of ovarian stem cells. Although the mrna expressions of Notch1, Hes1 and Hes5 were also observed to be increased, there was no significant difference in the expression of these genes between the control group and the Zuo GUI pill groups

(P>0.05), indicating that Zuo GUI Pill did not significantly regulate the transcription level of these specific genes.

5. Ameliorating Oxidative Stress

oxidative stress (OS) is a state of imbalance between oxidative and antioxidant systems in the body, which leads to excessive production of oxidative intermediates such as ROS, NOS, NO, and so on, triggering inflammatory response and cell apoptosis [25, 26]. As an important member of the histone deacetylase family, Silent mating type information regulation 2 homolog-1 (Sirt1) mediates a variety of cell fates by regulating p53 activity [27]. Sirt1 mediates p53 deacetylation and inhibits the transcriptional activity of p53 through a NAD^+ -dependent mechanism, and then participates in cell survival, senescence, proliferation and apoptosis by regulating tumor suppressor and DNA repair pathways [28]. With the increase of P53 expression, the expression of P21 is also increased, which is related to cell division cycle, senescence and apoptosis [27]. Therefore, SIRT1/P53/P21 pathway is the basic pathway for cell senescence and death. Moreover, some studies have shown that traditional Chinese medicine formula can block the oxidative stress-induced ovarian granulosa cell senescence pathway by activating SIRT1 and inhibiting the expression of p53/p21 [29]. growth differentiaction factor 9 (GDF-9) plays an important role in promoting follicle development, oocyte maturation and fertilization in theca cells, oocytes and granulosa cells in the form of autocrine and paracrine [30, 31]. brain derived neurotrophic factor (BDNF), an indicator of growth biochemical factors, functions as a trophic neuron and can improve the efficiency of oocyte maturation by regulating the gene expression of cumulus cells [32, 33].

Studies have found that Zuogi pill can improve the oxidative stress response in follicles by reducing oxidative stress indicators ROS, NO, NOS, regulating SIRT1/P53/P21 pathway, reducing the production of peroxide products, and protecting ovarian function. Moreover, it can improve the ovarian function damage caused by CTX by increasing the concentration of BDNF and GDF-9 in mouse ovaries [16, 34].

6. Promotes Ovarian Angiogenesis.

As a highly active organ of angiogenesis, the physiological function of the ovary significantly depends on adequate blood perfusion. Ovarian microcirculation disorders cause local ischemia and hypoxia, which can accelerate the process of cell apoptosis and follicular atresia, and eventually lead to ovarian function decline. Based on this, the exploration of angiogenesis mechanism can provide therapeutic strategies for improving the ovarian blood flow disorders in DOR. PLGF is a member of the Vascular endothelial growth factor (VEGF) family. PLGF and Flt-1 are widely distributed in various tissues and organs of the body. PLGF specifically binds to Flt-1 receptor and promotes functional angiogenesis by enhancing endothelial cell proliferation and chemotactic migration [35]. PLGF directly promotes angiogenesis and enhances the activity of other VEGF family members. This factor and its receptor Flt-1 are specifically expressed in reproductive organs and regulate local angiogenesis. Li Ruirui et al. [36] found that the positive rate of PLGF protein expression in female normal ovarian tissues was 18.75%. Meng Fanmou et al. found that Flt-1 existed in rat ovary and could increase vascular permeability and promote the formation of ovarian capillaries [37]. PLGF and its receptor Flt-1 play an important role in promoting angiogenesis and improving the function of reproductive organs.

Some studies have shown that Zuogui pill containing serum can significantly promote the proliferation of human umbilical vein endothelial cells (HUVEC) and enhance the length, diameter, area and tube formation index of endothelial tubules in three-dimensional model of angiogenesis in vitro. In addition, Zuogui pill can also promote angiogenesis by increasing the levels of serum E_2 and VEGF and up-regulating the expression of ovarian PLGF and Flt-1 protein. Improved ovarian blood supply [38, 39].

7. Summarization and Prospect

This article reviews the mechanism of Zuogui pill in the treatment of DOR, and finds that Zuogui pill can improve DOR through multi-target and multi-link regulation. For example, although network pharmacology predicts that Zuo GUI pill and DOR are involved in multiple signaling pathways, current experimental studies have only confirmed that SIRT1/P53/P21 and Notch signaling pathways are related to them, and current research mainly focuses on a single pathway, and future research on the diversity of pathways needs to be strengthened. The interaction between each pathway and the potential intermediate transmitters were further explored. Existing studies have confirmed that inflammatory injury and ferroptosis are involved in the pathological mechanism of diminished ovarian reserve (DOR), and traditional Chinese medicine can improve ovarian function by inhibiting ovarian ferroptosis [40, 41]. Whether Zuogui pill can improve the pathological process of DOR by inhibiting ovarian ferroptosis has not been clarified, and this mechanism needs to be used as a new direction for its research. In summary, a series of experimental studies of Zuogui Pill have confirmed its specific mechanism of action in the treatment of DOR, and the clinical treatment with western medicine and moxibustion has also received satisfactory effects [42-44]. Therefore, Zuogui pill can be better popularized in clinical practice and benefit more patients.

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