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Progress in TLR4/NF-κB Pathway Regulation by Traditional Chinese Medicine for the Treatment of Polycystic Ovary Syndrome

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Abstract: ***Polycystic ovary syndrome (PCOS) is a common reproductive endocrine disease in women, affecting women's fertility and cardiovascular system. Due to the complexity of its pathogenesis, there are many adverse reactions commonly used in clinical treatment, and the therapeutic effect is short-lived and temporary. TLR4/NF-κB pathway is a classical signaling pathway related to inflammation, and the effective components of TCM compounds and TCM monomers have been proven to reduce the levels of various inflammatory factors in patients by regulating the TLR4/NF-κB pathway, so as to achieve the effect of PCOS. The potential value of TCM in prevention and treatment of PCOS based on TLR4/NF-κB pathway was revealed. This article aims to summarize the recent studies on the improvement of PCOS symptoms by regulating the expression of TLR4/NF-κB pathway by Chinese medicine, so as to further study the mechanism of PCOS prevention and treatment by this pathway under the regulation of Chinese medicine, so as to open up new ideas for the development of new drugs targeting this pathway and clinical research.***

1. Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disease in women of reproductive age [1]. It presents with hyperandrogenemia, ovulation disorders and polycystic changes of the ovaries, usually accompanied by irregular menstruation, infertility, acne and insulin resistance [2]. At the same time, the incidence rate of gestational complications such as gestational diabetes, preeclampsia and stillbirth is also higher than that of women without the disease [3]. According to the Rotterdam diagnostic criteria, the incidence of PCOS in women of childbearing age is 5% to 20% [4]. Due to the complexity and heterogeneity of its pathogenesis, the current treatment regimen has not achieved satisfactory results [5]. Conventional Western medicine treatment is mainly to improve symptoms, but there are many adverse reactions, narrow scope of application and other problems. The Chinese medical treatment of PCOS has a long history and rich experience. According to its different manifestations, traditional Chinese medicine divides PCOS into "infertility", "irregular menstruation" and other categories. Traditional Chinese medicine believes that the pathogenesis of polycystic ovary syndrome lies in the deficiency of the essence and the deficiency of the spleen and kidney. The deficiency of the essence is mainly due to the pathological factors such as phlegm dampness, blood stasis and liver depression. Most doctors regard "tonifying kidney and spleen", "drying dampness and resolving phlegm" and "soothing liver and regulating qi" as the treatment methods for PCOS [6].

Previous studies have found [7] that the level of inflammatory factors in PCOS patients is increased, indicating the existence of low-grade chronic inflammation. TLR4 belongs to a classical anti-inflammatory pathway, which promotes the release of inflammatory factors by activating nuclear factor NF-κB, and is closely related to the pathogenesis of PCOS. In this paper, the intervention of TLR4 and NF-κB related pathways with traditional Chinese medicine was summarized, in order to provide a theoretical basis for further elucidate the molecular mechanism of traditional Chinese medicine in the treatment of PCOS, and to provide a reference for exploring the potential treatment of PCOS.

2. TLR4, and NF-κB

The Toll-like receptor family is an important class of protein molecules involved in non-specific immunity, which can recognize molecules with conserved structure from microorganisms with high conserved specificity. Studies have shown that Toll-like receptors (TLRs) play an important role in the pathogenesis of autoimmune diseases. TLRS belong to a family of pattern recognition receptors (PRRs) that recognize a wide range of pathogen-associated molecular patterns (PAMPs). The structure of tlr is characterized by cytoplasmic Toll/ interleukin-1 receptor (TIR) domains and extracellular leucine-rich repeats. tlr is a type I transmembrane protein located on various cell membranes. There are two main groups based on their location; The extracellular group refers to the group located on the plasma membrane, while the intracellular group is located in the endosomal chamber responsible for recognizing nucleic acids. They are released by host cells and trigger various intracellular pathways, leading to the production of pro-inflammatory cytokines, chemokines, and the expression of co-stimulatory molecules to protect against invading microorganisms [8]. Toll-like receptor 4, the first TLR to be discovered, is a type I transmembrane protein that is expressed in various tissue cells, including monocytes and macrophages. It plays a central role in the innate immune response. It not only binds to exogenous ligands on the surface of the cell membrane, but also interacts with intracellular ligands to initiate a complex intracellular signaling cascade. TLR4 activates the transcription factors NF-κB and AP-1 through the adaptor protein MyD88, thereby promoting the upregulation of pro-inflammatory cytokines [9].

NF-κB is an inducible transcription factor that can regulate the expression of a large number of genes, and is mainly

involved in the regulation of immune inflammatory response and cell differentiation [10]. Members of the NF-κB family have five proteins in mammals, divided into two subgroups [11], with RelA (p65), RelB, and c-Rel forming the first group. The second group consists of NF-κB1 (p50) and NF-κB2 (p52). The NF-κB subunit forms homologous or heterodimers, each with a unique function. P105 and p100 undergo proteolytic changes before reaching the mature states of p50 and p52, respectively. Their N-terminal has a highly conserved Rel homology region (RHR), and these proteins act as dimerizing transcription factors [12], regulating gene expression and influencing various biological processes. NF-κB finely regulates the expression of different genes in the form of different dimers. It has been found that the signal transduction pathway of NF-kB is activated mainly in the following two ways: (1) stimulated by pro-inflammatory cytokines, such as tumor necrosis factor (TNF) -α and interleukin (IL)-1, leading to phosphorylation of IκB by IκB kinase (IKK) complex [13]; (2) Activation of TNF family ligands, leading to phosphorylation of IKKα [5].

3. Relationship between TLR4, NF-κB and PCOS

Patients with PCOS have a chronic, low-grade inflammatory state, often accompanied by ovulation disorders. TLR4 is a transmembrane protein receptor involved in the body's non-specific immunity, initiating the transcription and translation of various inflammatory mediators through various signaling pathways. TLR4 activates the NF-κB signaling pathway by recognizing and transducing lipopolysaccharide (LPS). Activation of NF-κB leads to the production of various inflammatory cytokines, such as Tumor Necrosis Factor-α (TNF-α), Interleukin-1β (IL-1β), and Interleukin-6 (IL-6), Which in turn drives the aggregation of inflammatory cells. The inflammatory response activates microglia and astrocytes, prompting them to release inflammatory mediators. In turn, the inflammatory response can affect the normal function of mitochondria, causing ROS accumulation and damage to the function of the antioxidant enzyme system, and then synthesizing a large number of oxygen free radicals, thereby causing lipid peroxidation damage to ovarian tissue [14]. At the same time, the follicle is the basic functional unit of oocyte genesis and development, and the cytokines secreted by granulosa cells are the main components of the follicle microenvironment. Under physiological conditions, adequate inflammatory stress is necessary for normal follicle development and ovulation, and contributes to the growth and development of oocytes. However, excessive inflammatory stimulation will limit the growth of oocytes, resulting in premature follicle atresia, and even ovulation disorders. NF-κB signaling pathway can damage islet beta cells by promoting inflammatory response, resulting in absolute or relative insufficiency of insulin secretion, resulting in glucose metabolism disorders [15]. Hyperglycemia can activate the NF-κB signaling pathway in monocytes and promote systemic inflammatory response, thus aggravating the impaired function of islet beta cells in PCOS patients [16]. 50% to 80% of PCOS patients are associated with obesity, and abdominal obesity is the main problem. Excessive fat accumulation increases the number of macrophages in adipose tissue of PCOS patients, leading to the dysfunction of adipocyte metabolism and activation of NF-κB signaling pathway. At the same time, the increase and activation of macrophages may lead to the excessive release of tissue lysase, thus accelerating the inflammatory response [17].

4. TCM can Improve and Treat PCOS by Regulating TLR4 and NF-κB Related Pathways

PCOS affects women's life from various aspects such as "menstruation, fetus, childbirth" and postmenopausal type 2 diabetes, hypertension, and increased incidence of endometrial cancer. TCM compounds and their active components can target TLR4 and NF-κB signaling networks, regulate insulin resistance, glucose and lipid metabolism, autophagy and other pathological mechanisms in ovary, pancreas and other parts to improve PCOS.

4.1 TCM Compounds Act on TLR4 and NF-κB Related Pathways

4.1.1 Bushen Huatan prescription

Bushen Huatan prescription is the fifth batch of famous Chinese medicine instructor Hou Lihui's experience prescription, the main components of orange peel, poria, pinellia, bamboo ru, attrition, Cornus officinalis, Xianling spleen, Morinda, Fritillaria thunbergii, saponaria sinensis, incense, raw hawthorn and so on for the treatment of kidney deficiency phlegm-dampness syndrome PCOS has significant effect. Some scholars have found [18] that Bushen Huatan prescription combined with acupoint embedding can effectively improve the level of sex hormones, sugar and lipid metabolism in PCOS patients with kidney deficiency phlegm-dampness syndrome. The mechanism may be to reduce the level of inflammatory factors by inhibiting the activation of TLR4/NF-κB signaling pathway, so as to achieve therapeutic purposes.

4.1.2 Guizhu Yikun Formula

Wang Yang [19] et al. explored the therapeutic effect of Guizhu Yikun formula combined with fecal bacteria transplantation on PCOS rats through experiments, and the experimental results showed that: Guizhuykun formula combined with fecal bacteria transplantation can significantly improve testosterone, LH/FSH, IL-18, TNF-α and other indexes ($p<0.05$), which may be related to the down-regulation of TLR4/NF-κB signaling pathway and the improvement of chronic inflammation

4.1.3 Cangfu Daotan Decoction

Phlegm-dampness syndrome is often witness type of PCOS. Cangfu Daotan Decoction is a common prescription for the treatment of phlegm-dampness polycystic ovary syndrome. It comes from Ye's Female Branch. In its composition, atractylotis has the function of strengthening spleen and dampness, fructus aurantii and Xiangfu for Qi width, and opening up phlegm in chest and flank, Dannan Xing for dampness and phlegm removal, orange peel, poria cocos and licorice have the function of dampness and phlegm removal. Clinical studies have proved that Cangfu Daotan Decoction

has significant curative effect on spleen deficiency phlegm-dampness PCOS, and can effectively improve infertility in patients with PCOS by regulating endocrine conditions and reproductive metabolism of patients [20]. HMGB1 is closely related to the occurrence of IR pathological process [21]. Wu Xiaozhen [22] believed that the expression of HMGB1 was down-regulated under the influence of Cangfu Daotan Decoction, thus inhibiting the activation of TLR4/NF-κB signaling pathway and reducing the release of inflammatory factors.

4.1.4 Added peony and licorice soup

Shaoyao and glycyrrhizin decoction was first found in the Treatise on Febrile Diseases written by Zhang Zhongjing, a famous doctor in the Han Dynasty. Zeeland inhibits the release of inflammatory factors [23]. Chang Zhuangpeng [24] et al. added Zeilan flavor to Shaoyaogao Decoction and found through experiments that the added flavor of Shaoyaogao decoction could significantly reduce the expression of NF-κB and related proteins in the ovarian tissue of letrozole-induced pcos rats and the levels of downstream inflammatory factors such as TNF-α, so as to improve the function and morphology of polycystic ovary.

4.1.5 Huayu Jiangzhuo Formul

Huayu Jiangturbidity formula can significantly reduce the relative expression levels of TLR4, MyD88, and NF-κBp65 proteins in ovarian tissue of rats, reduce the levels of T, LH, LH/FSH in serum of rats [25], improve ovarian function and correct ovarian hormone disorders [26].

4.1.6 Erchen Decoction

Animal experiments have proved that Erchen Decoction can effectively improve the symptoms of insulin resistance, disorder of glucose and lipid metabolism, and polycystic changes of ovary in pcos rats [27-28]. Shi Shumei et al. [29] established the model by trozole combined with high-fat diet, and determined that the levels of serum hormones, glucose and lipid metabolism indexes and inflammatory factors of the rats in Erchen Decoction group were lower than before, and follicles at various developmental stages were visible in the ovaries, which may be related to the inhibition of NF-κB and NLRP3 mediated inflammatory response by Erchen Tang.

4.2 The Active Components of Traditional Chinese Medicine Act on TLR4 and NF-κB Related Pathways

4.2.1 Ligustrazine

Ligustrazine is one of the effective components of ligustrum chuanxiong. The ancient book "Materia Medica Hui Yan" once recorded: Chuanxiong is the qi medicine in blood. Some scholars have found that ligustrazine has a certain anti-inflammatory effect and can reduce body damage [30]. Ligustrazine can reduce the phosphorylation of NF-κB by up-regulating the expression of PPAR-gamma protein, thereby reducing the inflammatory response of ovarian tissue in PCOS rats and improving ovarian function [31].

4.2.2 Puerarin

Puerarin is a kind of natural isoflavone carbon glycoside from plants, which can be extracted from pueraria root. It has the effects of lowering blood lipid, lowering androgen and reducing insulin resistance level [32-33], and can be used to treat neuropathic pain [34], cardiovascular and cerebrovascular diseases, Alzheimer's disease, diabetes and other diseases. Puerarin can inhibit the activation of TLR4/NF-κB pathway and improve TNF-α, IL-6, FINS, LH, IR and other indexes in PCOS rats.

4.2.3 Berberine

Berberine, also called berberine hydrochloride, can be extracted from Coptis and other plants, and belongs to a quaternary ammonium alkaloid. Studies have confirmed [35] that berberine can improve insulin resistance, is an insulin sensitizer [36], and can down-regulate the activity of NF-κB [37], which is related to the fact that berberine inhibits the secretion of inflammatory factors IL-1β and INF-γ, and effectively regulates the imbalance of intestinal flora. Berberine inhibits NF-κB activity, improves the inflammatory state of ovarian tissue [38], and reduces the number of atretic follicles and cystic follicles.

4.2.4 Artesunate

Artemisinin, also called artemisinin, has a unique sesquiterpene structure. As one of the derivatives of artemisinin, artesunate not only has remarkable effects in the treatment of malaria, but also has certain effects in anti-tumor and anti-inflammatory aspects, which can inhibit the activation of p38MARK/NF-κB pathway and inhibit the cellular inflammatory response induced by lipopolysaccharide [39]. Its effects on improving ovarian morphology, function, insulin sensitivity, and promoting sex hormone recovery may be related to reducing the expression of inflammatory factors by inhibiting the NF-κB inflammatory pathway [40].

4.2.5 Sea buckthorn flavonoids

Sea buckthorn has the homology of medicine and food, and has the effect of promoting blood circulation, dispelling stasis, relieving stagnation and relieving cough and phlegm. Its main functional components are flavonoids. Sea-buckthorn flavonoids have been proved to have the functions of lowering blood lipids, anti-oxidation, enhancing immunity, inhibiting bacteria and lowering blood sugar [41]. Wang Yujing [42] et al. found that flavonoids of sea buckspur can reduce the contents of ovarian index, FBG, HOMA-IR, LH, T, TNF-α, IL-6 and CRP in PCOS rats, and at the same time reduce the expression of NF-κBp65 mRNA and TLR4, MyD88 and p-NF-κBp65 protein. Relieve the pathological morphology of ovarian tissue.

4.2.6 Quercetin

Quercetin is widely found in angiosperms and has the effects of lowering blood lipids, lowering blood sugar and anti-inflammatory. Wang Z [43] et al. made a model of DHEA and injected quercetin into the stomach for 28 days. The results showed that quercetin significantly reduced the levels of insulin, interleukin1β, IL-6 and tumor necrosis factor α in

the blood, and also significantly reduced the translocation of NF-κB in granule nuclei of insulin-resistant PCOS rats. Treatment inhibited the expression of inflammation-related genes, including nicotinamide adenine dinucleotide phosphate oxidase subunit p22phox, oxidized low density lipoprotein and toll-like receptor 4 in ovarian tissue, improved IR, and had a good therapeutic effect on PCOS rats. The mechanism of quercetin may be related to inhibition of toll-like receptor /NF-κB signaling pathway and improvement of inflammatory microenvironment in ovarian tissue of PCOS rats.

4.2.7 Resveratrol

Resveratrol can be antibacterial, antioxidant, anti-aging, anti-cardiovascular disease, natural sources of resveratrol are knotweed and grapes. Brenjian S [44] et al. found that by inhibiting NF-κB factor and gene products regulated by NF-κB signaling pathway, resveratrol can reduce serum levels of IL-18, NF-κB and other inflammatory factors in PCOS patients.

4.2.8 Cryptotanshinone

Cryptotanshinone is a diterpenoid quinone compound derived from Salvia miltiorrhiza. Its role in protecting ovaries and improving fertility may be related to the down-regulation of TLR4 and NF-κB/p65 expression levels [45].

4.2.9 Soy isoflavones

The structure of soybean isoflavones is similar to estrogen, and the natural soybean isoflavones are mainly divided into free glycosides and bound glycosides. Its role is rich, in addition to affecting the secretion of hormones, protein synthesis, metabolic biology and growth factor activities also play a significant impact. It can inhibit the phosphorylation level of NF-κBp65, down-regulate the expression of NF-κBp65, p-NF-κB p65 and p-IκBα, increase the level of E2 and FSH, and up-regulate the expression of NF-κB inhibitor IκBα gene, so as to achieve anti-inflammatory, improve the estrus cycle, restore ovarian morphology and serum sex hormone level. This is the purpose of treating PCOS [41].

4.3 Acupuncture Treatment

Belt Vessel failure is one of the pathogenesis of PCOS that cannot be ignored [46]. "Fu Qing main female Branch" said, "but the belt vessel is tied between the waist and the umbilicus, and almost to the Yin place", at the same time, "pulse classic" recorded in the belt vessel master "the woman does not come to the moon water, never follow the Fu down, Yin cold, no children. " Experimental studies have shown that body fat parameters, insulin resistance index and NF-κB signaling pathway in hypothalamus of rats were improved after the intervention of "Dai mai" point with electroacupuncture [47]. In clinical PCOS-IR patients, the pulse-bearing pivot is often damaged, the spleen and stomach are removed and the accumulation of phlegm leads to dampness. Phlegm and dampness circulate through the lower cell palace, blocking the cell portal and causing the kidney water to reach the cell palace, and the channel water is blocked. Electroacupuncture pulse-bearing intervention provides another way of thinking for the clinical treatment of this kind of patients.

5. Summary

With the development of modern society, the incidence of PCOS is gradually increasing, but the treatment methods for PCOS have not made significant development and innovation. Compared with Western medicine, Chinese medicine has the advantages of multi-target, multi-pathway and less adverse reactions, and has become a hot spot in the prevention and treatment of polycystic ovary syndrome. In recent years, a large number of animal experiments have shown that Chinese medicine monomer and Chinese medicine recurrence have good effects on the treatment of polycystic ovary syndrome. In this paper, we summarized the mechanism of TLR4/NF-κB signaling pathway regulated by TCM compounds and TCM monomers, and provided ideas for further exploring the specific molecular mechanism in the future, in order to promote the prevention and treatment of PCOS, a complex disease.

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