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The Mechanism and Treatment Research Progress of Metformin in Treating Endometriosis

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Abstract: Endometriosis is a common disease in women of childbearing age, and its specific pathogenesis is not yet clear. The clinical pharmacological treatment mainly involves the continuous use of progestins, GnRH analogs, danazol, aromatase inhibitors, and other drugs. However, some studies have shown that various medications have limitations in their application for the treatment of endometriosis. Metformin is a widely used insulin sensitizer for treating type 2 diabetes. Due to its pleiotropic effects, metformin is now also applied in the treatment of endometriosis. It mainly works by activating the adenosine monophosphate-activated protein kinase (AMPK) signaling pathway, inhibiting interleukin (IL)-1-mediated IL-8 secretion, and reducing the expression of the anti-apoptotic gene Bcl-2. Metformin's use in treating endometriosis has a unique mechanism of action, without serious side effects, making it an effective anti-inflammatory and anti-proliferative drug. This article aims to explore the mechanism of action of metformin in endometriosis and the possibility of drug treatment.

1. Introduction

Endometriosis (EMT) is a common gynecological disease that mainly occurs in women of childbearing age, typically between 25 and 45 years old. It affects approximately 10% of women of reproductive age globally, with the prevalence among infertile women reaching 20% to 50%[1]. The exact causes and pathogenesis of EMT are still not fully understood. Several theories exist regarding the pathogenesis of EMT, including the implantation theory (retrograde menstruation, iatrogenic implantation, lymphatic and venous spread), the coelomic metaplasia theory, the induction theory, as well as interactions at hormonal, genetic, immunological, and environmental levels[2][3]. Traditional treatments for endometriosis mainly include surgery and drug therapy. However, traditional drug therapy only provides temporary relief of symptoms, and the drugs are often expensive with many side effects[4]. Metformin, as an oral hypoglycemic medication, has been clinically practiced in China for over 30 years[5]. Recent studies have found that Metformin can significantly reduce the incidence of tumors in diabetic patients[6]. In the pathogenesis of endometriosis, the "retrograde menstruation theory" is the most prominent, which elucidates the

similarities between endometriosis and malignant tumors in certain aspects such as progressive and invasive growth, estrogen-dependent growth, tendency for recurrence and metastasis[7]. Epidemiological and multiple clinical trial data indicate that long-term use of metformin has a preventive and adjuvant therapeutic effect on lung cancer, breast cancer, and gynecological malignancies[8]. Therefore, this article analyzes the mechanism and current diagnosis and treatment status of metformin in endometriosis.

2. The mechanism of metformin in endometriosis

2.1 Activating the adenosine monophosphate-activated protein kinase (AMPK) signaling pathway reduces estrogen synthesis: inhibiting the expression of steroidogenic acute regulatory protein (StAR) and P450 aromatase mediated by prostaglandin E2 in ectopic endometrial stromal cells, thereby reducing estrogen synthesis[9,10]

AMPK is a serine/threonine protein kinase that plays a crucial role in regulating cell metabolism, growth, and survival, serving as a primary enzyme in modulating cellular energy homeostasis[10]. Metformin can trigger the phosphorylation of mitogen-activated protein kinase (MEK)/extracellular signal-regulated kinase (ERK) [9][11], thus activating the AMPK signaling pathway and inhibiting prostaglandin E2 (PGE2) in ectopic endometrial stromal cells. PGE2 stimulates the transcription of StAR by enhancing the binding of the cyclic adenosine monophosphate response element-binding protein (CREB) and its co-activator CRTC2 to the StAR promoter region. This, in turn, activates the binding site of the cyclic adenosine monophosphate response element (CRE), increases AMPK phosphorylation to prevent the nuclear translocation of CREB-regulated transcription coactivator 2 (CRTC2), and weakens the association between CREB and CRTC2 as a transcription complex[10]. PGE2 can induce the nuclear translocation of CRTC2 and enhance its association with CREB, forming a transcription complex that binds to the StAR promoter region. PGE2 can also mediate the expression of P450scc, HDS3B2, and HSD17B1[9], inhibit the interaction between CREB and its co-activator CRTC2, and prevent them from binding to the StAR promoter region. Research has shown that metformin treatment can disrupt the association between CRTC2 and CREB for 12 to 48 hours, without altering the baseline levels of these two proteins[9]. Therefore, metformin alleviates endometriosis by triggering the AMPK signaling pathway, PGE2-mediated expression of StAR and P450 aromatase, etc., to achieve reduction of estrogen synthesis.

2.2 Metformin also reduces the secretion of interleukin (IL)-8 mediated by interleukin-1, decreases aromatase activity, and inhibits the proliferation of ectopic endometrial cells.

Inflammatory responses play a significant role in the development and progression of endometriosis[12]. Studies have shown that metformin can inhibit inflammation[13]. IL-8 plays a significant regulatory role in the inflammatory process[14], and the levels of IL-8 in the peritoneal fluid of patients with endometriosis are elevated[15]. Metformin can inhibit the production of IL-8 induced by IL-1β. According to a study by Takemura Y[13], human embryonic stem cells incubated with metformin showed a statistically significant dose-dependent reduction in IL-8 production induced by IL-1β. Metformin appears to exert anti-inflammatory effects by reducing the secretion of pro-inflammatory cytokines in specific cell types. It can inhibit the release of IL-8 in human adipose tissue[16] and suppress the release of IL-6 and IL-8 induced by IL-1β in human vascular endothelial cells. However, at the same dosage, metformin can inhibit the secretion of IL-8 in embryonic stem cells induced by IL-1β but does not inhibit the secretion of IL-8 in ectopic endometrial stromal cells[17]. As is well known, endometriosis is an estrogen-dependent disease, and the local production of estrogen within endometriotic tissues is a significant factor in the

pathogenesis. Research indicates[18] that abundant expression of aromatase can lead to an increase in local estrogen production in endometriotic tissues, suggesting that aromatase is responsible for local estrogen production. Aromatase inhibitors, by inhibiting the conversion of androstenedione to estrone, thereby suppressing follicle-stimulating hormone in granulosa cells, have also become one of the treatment options for endometriosis. This combined therapy primarily causes regression of endometrial implants in rodents through anti-estrogenic and anti-inflammatory effects, aiming to reduce the proliferation of ectopic endometrial cells.

2.3 It can lower the expression of the anti-apoptotic gene Bcl-2, increase the expression of the pro-apoptotic genes Bax and p53, restore the balance of the apoptotic regulatory system within the eutopic endometrial cells, thereby promoting cell apoptosis.

Cell apoptosis is an important factor for the cyclical shedding and sustained stability of endometrial tissue, and it is precisely due to the increased anti-apoptotic activity of endometrial cells that ectopic endometrial tissue is able to implant outside the uterine cavity and survive[19]. The most studied apoptotic regulatory proteins in EMT are Bcl-2, Bax, and P53. Bcl-2 is an inhibitor of the intrinsic apoptotic pathway. Shan[20] found that activation of the ERK signaling pathway in EMT lesions can lead to Bcl-2 activation, thereby reducing EMT cell apoptosis and increasing cell survival. Lyu S and others[21] discovered that EMT patients exhibit significantly increased expression of the anti-apoptotic gene Bcl-2 protein, while the expression of the pro-apoptotic genes Bax and P53 is significantly lower compared to non-EMT individuals, resulting in an elevated Bcl-2/Bax ratio. The imbalance in the expression of the apoptotic regulatory proteins leads to a significantly lower apoptosis rate in endometrial cells of EMT patients compared to normal endometrial cells. This places them in a state resembling a special immunological function, where ectopic endometrial cells cannot be cleared normally, leading to the formation of ectopic lesions and the development of symptoms. However, metformin can reduce the expression of the anti-apoptotic gene Bcl-2, increase the expression of the pro-apoptotic genes Bax and p53, restore the balance of the apoptotic regulatory system within eutopic endometrial cells, thereby promoting cell apoptosis[22]. ELGENDY M's research[23] has found that metformin dephosphorylates protein phosphatase 2A (PP2A) to activate GSK-3β (glycogen synthase kinase-3 beta), reduces the levels of MCL-1 (a member of the Bcl-2 family), and induces apoptosis in tumor cells. Through research, Zheng Z et al.[24] found that metformin increases the expression of the pro-apoptotic protein Bax, elevates the Bcl-2/Bax ratio. LISMH research[24] discovered that metformin upregulates P53, stimulates the p53 signaling pathway, reduces the protein kinase B (AKT)/mTOR (mammalian target of rapamycin)/eukaryotic translation initiation factor 4E-binding protein 1 signaling pathway to induce cell apoptosis and cell cycle arrest, thereby inhibiting tumor growth[26]. Li et al.[25] observed that metformin decreases p53 expression levels, significantly induces apoptosis and cell death, and increases the phosphorylation of liver kinase B1 (LKB1), promoting p53 activation and AMPK, and inhibiting cell cycle progression[27].

3. The application of metformin in the treatment of endometriosis

3.1 Treatment of metformin combined with progestin

Deferiprone is a synthetic progestin commonly used in clinics, which can effectively reduce the endocrine stimulation of the ovary in patients with endometriosis, prevent endometrial hyperplasia and carcinoma due to high estrogen, accelerate the contraction of ectopic endometrial tissues and at the same time prevent new endometrial ectasia, and does not damage the normal endometrium of the body while having this effect, and does not inhibit normal ovulation in patients after the use of

the drug, which is especially suitable for patients with fertility requirements[28]. It is especially suitable for patients with fertility requirements. Peng et al.'s study and analysis of 1709 cases using dienogest to treat patients with endometriosis showed that compared to progesterone, dienogest can significantly alleviate dysmenorrhea, increase the pregnancy rate, and reduce the risk of adverse events, and that dienogest can lower the risks of endometriosis recurrence and elevated transaminase levels compared to GnRH-a[29]. It can be seen that dienogest has relatively mild drug side effects in the treatment of endometriosis, with good drug safety and tolerability. However, conservative treatment of ovarian endometriotic cysts with oral dienogest requires a longer treatment process. As scholars have found through research, it takes 17 months for ovarian endometriotic cysts to show significant shrinkage when treated with oral dienogest, and complete disappearance of the cysts may take up to 40 months[30]. Therefore, it is quite possible that poor early efficacy, prolonged treatment processes, and high costs may lead to decreased patient compliance. In comparison to the singular drug effects, Cai Wenjuan et al.[31] found that the combined use of dienogest with metformin yields better results. The two can work synergistically, not only effectively improving the patient's symptoms and accelerating recovery, but also reducing the recurrence rate and occurrence of adverse reactions, while also enhancing the inhibitory effect on estrogen, effectively reducing the levels of P, FSH, and E2 in the body.

3.2 Treatment of metformin combined with aromatase inhibitors

Uterine endometrial tissues of patients with endometriosis have relatively high expression of aromatase, and the estrogen content can also increase significantly[32]. Aromatase inhibitors have a significant effect on accelerating apoptosis of endometrial cells and can also inhibit estrogen synthesis. Aromatase inhibitors typically include steroidal and nonsteroidal types, commonly used in clinical practice such as letrozole, anastrozole, etc. Among them, letrozole is a nonsteroidal aromatase inhibitor with very high selectivity and relatively strong specificity, showing significant effectiveness in the treatment of endometriosis. Some scholars have shown through clinical studies that when letrozole is used alone to treat endometriosis for 3 months, over 50% of patients may develop functional ovarian cysts. Even after undergoing surgery for endometriosis followed by letrozole treatment for 2 months, about 24% of patients still experience functional ovarian cysts, along with elevated FSH levels detected after 3 months of postoperative treatment[33]. Therefore, it can be seen that the effect of using letrozole alone for patients with endometriosis is not very ideal, indicating that in practice, other drugs should be used in combination for adjuvant therapy. Some scholars have found through research that the combined treatment of metformin and letrozole for endometriosis can inhibit the secretion of androgens and improve clinical outcomes[34]. Researchers such as Tian Liang [35] found through their studies that the combined treatment of letrozole and metformin resulted in a better overall efficacy and improvement in pain severity compared to the control group using letrozole alone. The application of letrozole and metformin in combination for the treatment of endometriosis has shown significant effectiveness. There are also clinical studies with small sample sizes showing that the combination of metformin and letrozole for treating endometriosis is more effective in improving pain symptoms, reducing nodular lesions, and lowering the recurrence rate compared to using letrozole in combination with leuprorelin[22].

4. Side effects of metformin

While there are many benefits to using metformin, some of its side effects should not be overlooked. Some of the most common side effects of using metformin derivatives are gastrointestinal symptoms[36]. Due to long-term use leading to the accumulation of substances in the intestine, approximately 20-30% of patients may experience mild to moderate illness[37]. In the

study conducted by Ma et al.[38], 44% of patients experience symptoms of diarrhea, while nausea is reported in as high as 40% of cases. For common side effects, starting with a low dose, gradually increasing the dosage, taking immediate-release formulations with meals, or switching to extended-release formulations can help reduce gastrointestinal adverse reactions[5]. If chronic diarrhea progresses to the point where hospitalization is needed, it can manifest as symptomatic electrolyte abnormalities such as hypocalcemia, hypokalemia, hypomagnesemia, and hypophosphatemia. Most gastrointestinal symptoms can be alleviated by supplementing with vitamin B12[39]. The reason for this is that long-term use of metformin can lead to poor absorption of vitamin B12[40]. If patients suddenly develop anemia and/or peripheral neuropathy, this cause should also be considered[41]. It is recommended that patients with inadequate intake or absorption of vitamin B12 undergo annual monitoring of vitamin B12 levels before and after starting metformin treatment, and if deficient, appropriate supplementation should be provided. It is worth noting that a very rare complication of metformin is lactic acidosis, a life-threatening condition that can present with symptoms such as arrhythmia, dizziness, difficulty breathing, and significant diarrhea[42]. This condition may be induced by the accumulation of metformin in the body, although there is currently no definitive report establishing a causal relationship. It is relatively common in patients with heart or lung diseases, acute worsening of kidney function, or sepsis, but long-term use of metformin does not increase the risk of lactic acidosis in patients with normal liver and kidney function[12]. The incidence of lactic acidosis with the appropriate use of metformin is not significantly different from other antidiabetic treatments[43].

5. Conclusion

The foundation of treating endometriosis is the reduction of endogenous steroid hormone production[44]. However, the long-term use of individual drugs such as progestins, GnRH analogues, danazol, and aromatase inhibitors has gradually revealed their limitations. This may be related to factors such as their side effects, cost, delayed fertility, prolonged treatment duration, and high recurrence rates after discontinuation[45]. Metformin is a widely available drug with a low price, high safety profile, and relatively mild side effects. Adjunct medications can accelerate recovery, shorten treatment duration, and save overall costs. In patients with endometriosis, the combination treatment with metformin has been shown to alter the serum cytokine levels, increase the chance of conception, suggesting that metformin may have a beneficial role as an anti-endometriosis medication[46]. However, there is relatively limited existing research on the consequences of using metformin to treat endometriosis, and the results of these studies vary. Clinical trials are also scarce, and there is currently no clear evidence indicating its specific effect on endometrial mesenchymal transition (EMT) in the treatment of endometriosis. As a result, the widespread use of metformin in the clinical setting for treating endometriosis has yet to be established. Therefore, further research is needed to elucidate the role of metformin as an anti-endometriosis medication.

Author Contributions

F-X C was primarily responsible for literature retrieval and analysis, as well as drafting the initial manuscript. Q-Y W coordinated and supervised the collection and analysis of literature. W-H H reviewed and revised the manuscript, conducting critical reviews of important intellectual content. All authors contributed to the conception and design of the study. All authors read and approved the final manuscript.

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