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Review

Effect and mechanism of berberine against polycystic ovary syndrome

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ABSTRACT

For women of reproductive age, polycystic ovary syndrome (PCOS) is not a rare heterogeneous endocrine disorder and metabolic dysfunction. Menstrual problems, hyperandrogenism, polycystic ovary (PCO) and infertility often affect these women, and they are also prone to metabolic syndrome (MS) and insulin resistance (IR). As an isoquinoline alkaloid, Berberine (BBR) is the main effective component of *Coptis*. BBR, as a multi-target, multi-path plant extract, can interfere with the development of PCOS and relate to pathological process from many aspects, with less adverse reactions. It is mentioned in this review that BBR can alleviate IR, reduce the level of serum androgen, regulate lipid metabolism and moderate chronic inflammation. BBR is often used in combination with metformin, compound cyproterone (CPA) and other drugs, in order to achieve better therapeutic effect on PCOS.

1. Introduction

For women of childbearing age, polycystic ovary syndrome (PCOS) is not a rare heterogeneous endocrine disorder and metabolic dysfunction. PCOS is prone to be associated with hypersecretion of luteinizing hormone (LH), hyperandrogenism (HA), hyperinsulinemia, menstrual dysfunction, hirsutism, infertility, pregnant and neonatal complications. Other long-term health risks, metabolic complications and psychological problems can also be caused by PCOS, such as type II diabetes mellitus (DM2), cardiovascular disease (CVD) and endometrial carcinoma. Compared with non obese patients, obese patients with PCOS are more likely to suffer from these complications. Age is also a factor that increases the incidence of complications in PCOS [1]. PCOS is an endocrine disease caused by genetic and environmental factors. While, from the perspective of traditional Chinese medicine, PCOS refers to the excess caused by deficiency, and the syndrome of deficiency-excess combined is more common. The main disease is in the kidney, involving the liver and spleen, and there are many pathological changes, including kidney deficiency, liver stagnation, liver heat, spleen deficiency, phlegm-dampness and blood stasis.[2] Epidemiological survey shows that the incidence of PCOS is 5.6% in women of childbearing age in China [3].

At present, the treatment of PCOS advocates individualized comprehensive treatment according to clinical manifestations, patients' complaints and needs, and the degree of metabolic disorders. Periodic progesterone therapy, short acting compound oral contraceptive (COC) and estrogen progesterone cycle sequential therapy are often used to adjust the menstrual cycle, but COC may have adverse effects on insulin sensitivity, is dose-dependent, and it takes 3–6 months to take effect. Metformin is also widely used in clinic to adjust the metabolic disorder of patients with PCOS, but patients often have digestive tract symptoms such as severe malignant, abdominal pain, abdominal distention, diarrhea, and even renal functional damage and lactic acid poisoning. In addition, when letrozole is used to induce ovulation, there may also be adverse reactions such as fatigue or dizziness. It can be seen that most of the current treatment drugs only affect a certain pathological process of PCOS, and often accompanied by some adverse reactions. Therefore, it is urgent to find a multi-target, safe and effective drug, so as to provide a new idea for the treatment of PCOS.

Berberine (BBR) (Fig. 1), an isoquinoline alkaloid, is a main component of many commonly used medicinal plants, such as Coptis chinensis Franch [4]., Hydrastis canadensis L [5]. and Coptis japonica

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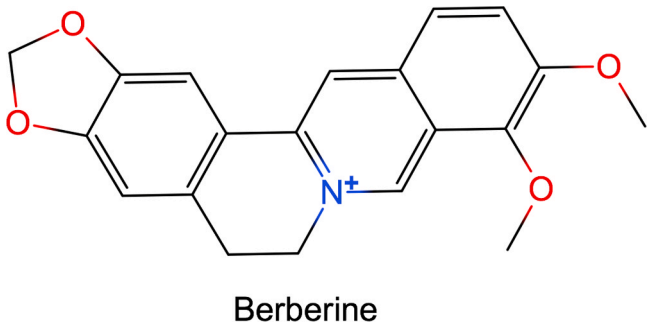

Fig. 1. The chemical structure of BBR.

Makino [6]. They are all generally believed to have the excellent effects of clearing heat, removing dampness, purging fire and detoxifying [7]. Most of the BBR used at present is synthetic in the form of chloride or sulfate. Berberine is a kind of yellow powder, bitter, slightly soluble in water and ethanol [8]. Studies [9,10] indicated that the absolute bioavailability of BBR was very low. The absorption rate of BBR in intestinal tract is poor. Most of BBR remain in gastrointestinal tract after oral administration, and then excrete with feces [11]. In addition, most of BBR absorbed by intestine was distributed in tissues, and the concentration of BBR in blood was low [12,13]. Modern pharmacological researches show that BBR has many functions, such as antibacterial, antiarrhythmic, dilating coronary arteries, lowering blood sugar and regulating blood lipid [14]. BBR can intervene the pathological process of PCOS by multiple pathways and targets [15]. Yu W et al [16]. found 15 potential targets of BBR, such as, aldo-keto reductase family 1 member C3 (AKR1C3), insulin receptor, estrogen receptor and tyrosine-protein phosphatase non-receptor type 1. Despite its low bioavailability, according to the published studies, BBR is expected to be a promising poly-pharmacological component or drug for PCOS.

In recent years, the research on BBR in the treatment of PCOS has increased gradually. We hope to provide basis and ideas for the further application of BBR in the treatment of PCOS through this review.

2. BBR can alleviate insulin resistance (IR)

Insulin resistance (IR) refers to a state of reduced insulin effect, that is, the sensitivity of insulin receptor to insulin is decreased. There is increasing evidences that IR is at the pathophysiological basis of PCOS [17]. Recent studies [18–21] have showed that at least a half of women with PCOS have IR. Hyperinsulinemia, a compensatory response to IR, may lead to HA in PCOS. Too much insulin in the body can induce the hypophysis to release a large amount of LH, as well as enhance the pulse amplitude of LH [22], by acting on the insulin receptor on the pituitary gland [23]. Excessive LH can inhibit the synthesis of sex hormone binding globulin (SHBG), increases the free androgen [24,25] which will affect the function of the ovary. Besides, insulin also promotes the arrest of preantral follicle development [26]. Of course, insulin can also stimulate the ovary to over secrete androgen [27,28], leading to HA. Hyperinsulinemia leads to the imbalance of myo-inositol and D-chiro-inositol in ovary, which impairs FSH signal, just as PCOS patients show [29,30]. Some studies, using insulin sensitizers (metformin 1.5 g/d for 6 m;[31] metformin 1.5 g/d for 8w [32]) to treat women with PCOS, found that their menstrual abnormalities and ovulation improved. All these evidences prove that IR is the pathophysiological basis of PCOS.

2.1. BBR activates insulin signaling pathway

A study of Chen CH et al [33]. found that in diabetic mice treated with BBR, hyperglycemia was reduced and the impaired glucose tolerance was improved, but insulin release or synthesis were not increased. It is reported that these effects of BBR may be due to its ability to inhibit the catalysis of protein tyrosine phosphatase 1B (PTP1B) and enhance the phosphorylation of insulin receptor and insulin receptor substrate-1 (IRS1) in 3T3-L1 adipocytes. A recent study [34] also confirmed that BBR can improve insulin sensitivity of PCOS patients by regulating IRS1 signaling pathway. In addition, BBR was demonstrated to activate the expression of key proteins in PI3K/Akt/GSK-3β insulin signaling pathway [35] and increase the phosphorylation of Akt. According to a recent report from Zhang N et.al, BBR inhibits mitogen activated protein kinase (MAPK) pathway [36]. MAPK, a critical signal transduction pathway, leads to an inseparable link between androgen biosynthesis and IR. As a result, the serum levels of testosterone(T) decreased while SHBG increased, and IR is reduced in rats with PCOS. In addition, BBR can increase the expression of peroxisome proliferator activated receptors (PPAR) α and γ in endometrium, improve the impaired glucose tolerance of PCOS rats, reduce the levels of LH, testosterone(T) and insulin, and improve the polycystic and adenomyosis like manifestations of ovary [37].

2.2. BBR can promote the utilization of glucose

Lee YS et al [38]. found that BBR can promote the transport and consumption of glucose in adipocytes and inhibit the differentiation of adipocytes. Another study [39] showed that oxygen-dependent glucose oxidation was inhibited and glycolysis was enhanced in PCOS obese women. These two ways can improve the utilization of glucose. A recent study by Li W et al [40]. confirmed that BBR promotes the ubiquitination of Sirtuin 3 (SIRT3), a major mitochondria NAD+ -dependent deacetylase [41], and then leads to AMP accumulation, which can activate AMPK signaling and further promoted glucose uptake. Similarly, BBR decreases the expression of phosphoenolpyruvate carboxykinase (PEPCK), glucose-6-phosphatase (G-6-Pase) and gluconeogenic genes in liver. BR can promote glucose uptake and further reduce PCOS pathology and IR values by up regulating the expression of Glut4 in ovarian [36]. As an insulin responsive glucose transporter, Glut4 also exists in the endometrium, which is mainly responsible for the basic sugar demand of cells. The reduced *Glut4* expression can be caused by PI3K/AKT activation and MAPK pathway suppression [36]. Thus, we concluded that BBR can alleviate IR by promoting the utilization of glucose in peripheral tissues.

3. BBR can reduce the level of serum androgen

The levels of various androgens in patients with PCOS are elevated, including T, pro-androgens androstenedione (A4) and dehydroepiandrosterone sulfate (DHEAS) [42]. In addition, the level of some androgen-activating enzymes such as 3β-hydroxysteroid dehydrogenase (3β-HSD) also increased [43]. The amount of androgen produced by the theca cells in patients with PCOS is 20 times higher than in normal people. Studies showed that not only the function of theca cells is changing in PCOS patients, but also the sensitivity of the pituitary gland for gonadotropin-releasing hormone increases dramatically, resulting in increased secretion of LH, which induces the follicular membrane cells to produce a large amount of androgens and hinder the maturation of follicles [44].

Excess androgens can be caused by IR and hyperinsulinemia as they lead to a decrease in SHBG levels, resulting in a subsequent increase in free androgens and adverse metabolic conditions [45,46]. A large number of studies have suggested that the key media for the occurrence and development of PCOS are androgens and their effects through androgen receptor (AR) [47]. Human [48] or animal [49] studies using AR antagonists or long-term blocking of AR signals [50] and studies in transgenic mouse models with silent androgen effects [51] have confirmed androgen-driven effects, particularly AR mediated neuroendocrine mechanisms.

BBR has been confirmed to reduce androgen levels in mice [52] and

women [53] with PCOS, which we believe are mainly mediated by the following two mechanisms:

3.1. BBR improve sex hormone binding globulin level

Maliqueo M et al.'s study [54] found that the expression of SHBG in endometrial stroma of PCOS patients was lower than that of control group. And as mentioned, IR in PCOS patients also causes a decrease in SHBG levels. Decreased SHBG levels increase androgen bioavailability, which in turn leads to ovarian pathology, anovulation, and the development of phenotypic features of PCOS [55]. Many experiments [14,53,56] have proved that BBR can increase SHBG levels, which is conducive to the stability of serum free androgen levels and improve the symptoms of hyperandrogenism in patients, such as acne and hairy.

3.2. BBR suppresses androgen receptor signaling

Contrary to the point above, another study [39] reported that there may not be any correlation, at least not obvious, between insulin resistance and changes in obesity and its effect on androgen levels. However, they pointed out that the antiandrogen effect of BBR may be achieved by acting directly on the ovary, as they found that BBR suppresses AR signaling. Li J et al. found that BBR induced AR protein degradation instead of mRNA expression [57]. Furthermore, Shen M et al [58]. found that the activation of AMP-activated protein kinase (AMPK) can directly decrease the amount of AR protein. Horman et al [59]. pointed out that AMPKα can be phosphorylated at Ser485/491 by Akt, thus making it difficult for AMPKα to be phosphorylated at Thr172, which reduces the activity of AMPK. As mentioned above, BBR can increase phosphorylation of Akt, so further research should focus on whether BBR can decrease AR protein by reducing the inhibition of Akt on AMPK activity.

3.3. BBR inhibits androgen synthesis

Another study [60] showed that BBR can reduce serum T by reducing the density of steroidogenetic acute regulatory protein (StAR) on the cell membrane of follicular membrane cells. StAR promotes the cholesterol outside the mitochondrial membrane to transport to the inner membrane. It is a key step for the production of steroids by theca cells, which determines the speed and quantity of androgen production. Besides, BBR could decrease intracellular androgen synthesis due to the suppression of aldo-keto reductase 1C3 (AKR1C3) activity [61]. AKR1C3 is a type 5 17-hydroxysteroid dehydrogenase which catalyzes the conversion of low active hormone precursors such as androstenedione and androsterone to highly active T and dihydrotestosterone in the last two steps of steroid synthesis.

From the perspective of gene expression, BBR can down regulate the expression of *CYP17a1* gene in ovary and up regulate the expression of *CYP19a1* in ovary [62]. The androgen related *CYP17A1* encodes the steroid producing enzyme cytochrome p45017 α - hydroxylase (P450c17), which is required for the synthesis of androgen and glucocorticoid from BBR. If the expression of the enzyme is down regulated, the androgen level can be reduced. The *CYP19A1* encodes aromatase. As a rate limiting enzyme, aromatase that promote the conversion of androgens to estrogens during the development of steroid. When the expression of the enzyme is up-regulated, its ability of catalyzing androgen to estrogen is improved, thus reducing the level of androgen. Therefore, BBR can improve the HA state of PCOS and regulate the hormone disorder by regulate the expression of these two genes, so as to achieve the therapeutic purpose.

4. BBR can alleviate abnormal lipid metabolism

Many aspects of PCOS will have negative effects if the patient is overweight or obese. For PCOS patients, central or visceral obesity may lead to more severe IR. This is due to the increase of free fatty acids (FFA) and the disorder of paracrine action of visceral depot [63]. It can also be said that obese people release more FFA through fat cells, especially abdominal fat cells, which affect the absorption of glucose by insulin-mediated skeletal muscle, fat cells and liver cells, reduce the sensitivity of liver insulin, and increase the output of liver glucose [64]. The cytokines secreted by brown adipose tissue (BAT) are the key to reduce IR and PCOS inflammation [65].

So alleviate abnormal lipid metabolism may improve PCOS and its related metabolic symptoms, as an important factor to cause PCOS.

4.1. BBR can inhibit synthesis of lipid

BBR can alleviate the abnormal lipid metabolism through various ways. Zhang M et al [66]. showed that BBR can reduce the synthesis of triglyceride (TG) via activating AMPK pathway, thus attenuate the lipotoxicity and improve the sensitivity of insulin. In addition, it has been confirmed that BBR can inhibit the synthesis of lipid in hepatocytes and thus significantly reduce the lipid storage in the liver [67]. It has also been confirmed that BBR can promote the proliferation of 3T3-L1 preadipocyte, but inhibit its differentiation to mature adipocytes and reduce the lipid content in adipocytes through PPAR signaling pathway [39] and up regulating the expression of *INSIG-2* [68].

4.2. BBR can increase lipid metabolism

Kong al et [69]. compared BBR with statins. The results showed that low density lipoprotein cholesterol (LDL-C), total cholesterol (TC) and TG decreased after treatment with BBR, and further revealed that BBR promoted the transcription of LDL receptor mRNA via activating extracellular regulated kinase (ERK) signal pathway. It can increase the expression of LDLR protein [70], increase the affinity of binding lipoproteins, mediate the cell to intake and metabolize lipoproteins protein to maintain the plasma LDL-C in a relatively stable level.

4.3. BBR can increase the synthesis of fat factors

Adiponectin is a protein secreted by adipocytes, which plays an important role in preventing IR. Gu W et al [71]. found that BBR can significantly increase the expression of adiponectin mRNA. However, Tu J et al [72]. reported that BBR does not increase the expression of adiponectin mRNA at transcriptional regulation level, but activates AMPK and increases the proportion of active adiponectin in the post translational regulation, thereby enhancing insulin sensitivity. Liu Y, et al [73]. found that in a certain range, BBR can also promote the expression of visfatin mRNA and protein synthesis of adipocytes in vitro. Visfatin is an adipokine related with obesity, and it is generated mainly by the visceral adipose tissue. Visfatin is able to improve the pathological process of PCOS, via maintaining glucose homeostasis and regulating genes related to oxidative stress (OS) as well as inflammatory response.

In conclusion, BBR may improve IR and lipid metabolism by reducing lipid synthesis, promoting lipid consumption and increasing fat factor, so as to regulate the endocrine system and reproductive function of PCOS patients.

5. BBR can alleviate chronic inflammation

Xiong YL.et al. found a large number of inflammatory cells in the ovary of PCOS patients, and the whole ovary showed a chronic and persistent inflammatory state [74]. XX Various cytokines produced by these inflammatory cells can induce apoptosis of granular cells and theca cells, which makes it difficult for these women with PCOS to produce dominant follicles.

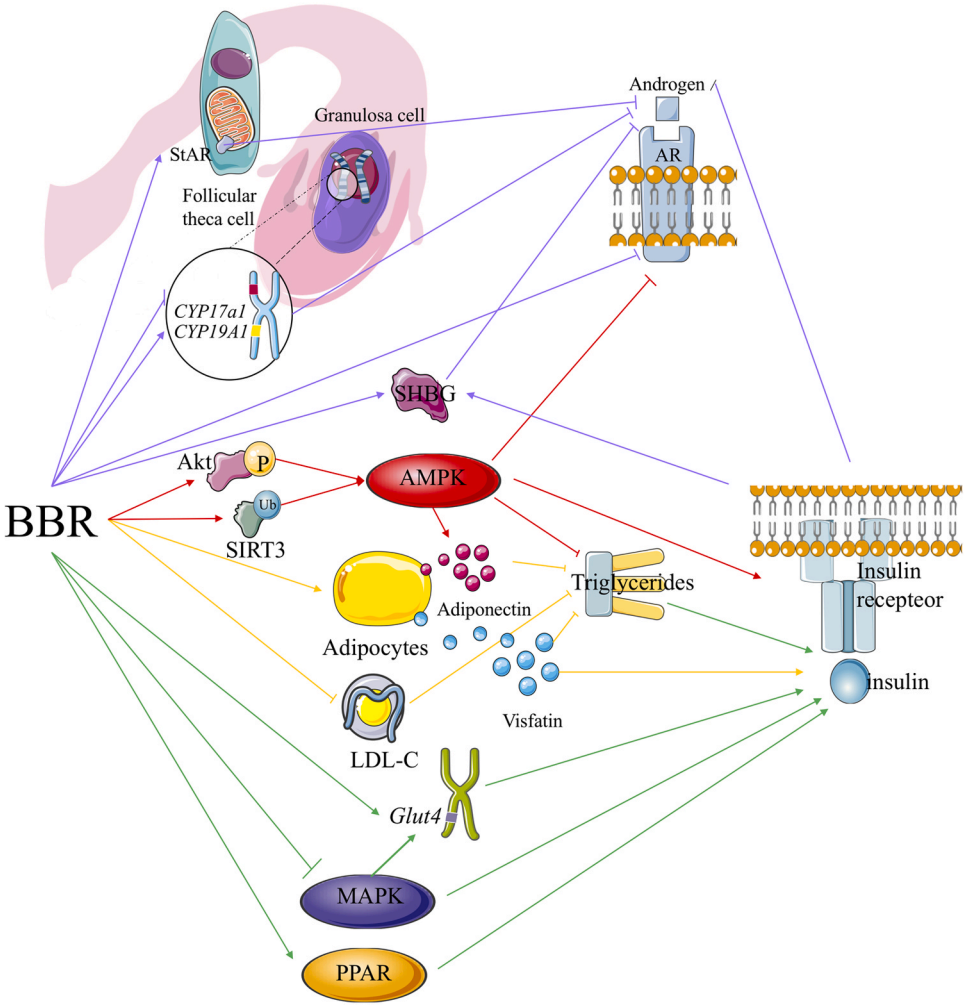

Fig. 2. The main mechanism of BBR against PCOS. The two main steps of BBR in the treatment of PCOS are to inhibit the binding of androgen to AR and promote the binding of insulin to insulin receptor. It can be seen intuitively from Fig. 2 that BBR works in various ways, and they all end up pointing to these two targets. In addition, effect of BBR on lipid metabolism is an intermediate link, and ultimately affects the binding of insulin and its receptor. Besides, increased insulin sensitivity can also inhibit the binding of androgen to AR. Abbreviations: LDL-c: low density lipoprotein cholesterol; AMPK: AMP-activated protein kinase; StAR: steroidogenetic acute regulatory protein; AR: androgen receptor; SHBG: sex hormone binding globulin; MAPK: mitogen activated protein kinase; PPAR: peroxisome proliferator activated receptors; SIRT3: Sirtuin 3;.

Furthermore, the peripheral tissues of PCOS patients also showed a chronic inflammatory state. Inflammatory cells and inflammatory factors were increased to a certain extent in patients' serum [75]. Some inflammatory factors, such as tumor necrosis factor - α (TNF - α) and interleukin-6(IL-6), can also increase the synthesis of androgen [74], aggravate IR, and even disturb hypothalamic-pituitary-ovarian axis [76]. So, scholars [77] have pointed out that chronic inflammatory response may be the intermediate link between PCOS and its related metabolic diseases.

Interestingly, BBR can reduce the expression of IL-6, CRP, TNF - α and other inflammatory factors, which are excessively produced in PCOS patients, so as to improve the damage of these inflammatory factors to ovarian tissue [75], and reduce the inflammatory state in PCOS patients. In addition, BBR can inhibit macrophage protein phosphorylation [3], lipopolysaccharide (LPS) induction of macrophages [78], and nuclear factor (NF) - κ B activity [79], and thus reduce the inflammatory response transmitters secreted by macrophages. Li YX and Li RH [80] have shown that BBR can reduce the level of prostaglandin E2 (PGE2) in local tissues of inflammatory response, and then reduce the inflammatory response mediated by PGE2. The mechanism of BBR anti-inflammatory activity also involves many kinds of cell kinases and signal transduction pathways, such as AMPK, MAPK [81].

In conclusion, BBR can reduce the production and release of inflammatory factors through various ways, so as to alleviate the chronic inflammatory response caused by PCOS, and achieve the role of treatment and prevention of related metabolic diseases (Fig. 2) (Fig. 3).

6. Other mechanisms of BBR in the treatment of PCOS

6.1. BBR can suppress oxidative stress

Patients with PCOS often have OS imbalance. Higher OS levels were found in PCOS patients with HA [82]. HA may improve the sensitivity of the body to OS, promote the release of various inflammatory factors induced by hyperglycemia, and directly or indirectly promote the occurrence of OS. OS can cause IR and compensatory hyperinsulinemia, stimulate the proliferation of ovarian theca-interstitial cells and the synthesis of T, reduce the secretion of SHBG in liver, and promote the occurrence of HA state [83]. BBR can enhance the expression of antioxidant enzyme activity by activating AMPK, PI3K/AKt pathway and p38 protein kinase pathway. For example, BBR can active superoxide dismutase (SOD), glutathione peroxidase (GSH-PX) as well as malondialdehyde (MDA) [84]. These antioxidants can protect the biological membrane of the body from the damage of free radicals, reduce the consumption of glutathione [39] and the generation of reactive oxygen species [85], thus inhibiting lipid peroxidation.

6.2. BBR can interfere with intestinal flora disorder

In recent years, studies [86,87] have shown that there is a close relationship between intestinal flora disorder and PCOS. A study [17] speculated that BBR can alleviate IR through the intervention of intestinal flora in patients with PCOS, so as to treat PCOS. For example, BBR can significantly reduce the proportion of *Firmicutes* and *Bacteroides* in

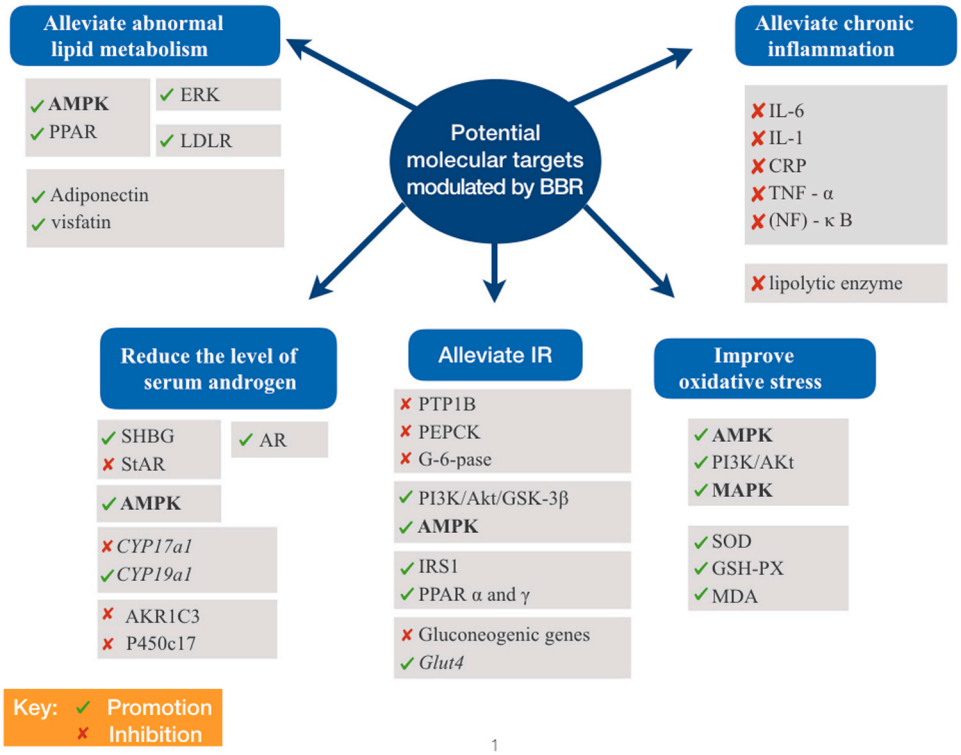

Fig. 3. Potential molecular targets modulated by BBR The potential molecular targets modulated by BBR can be summarized into five aspects as shown in this figure: alleviating IR, reducing the level of serum androgen, alleviating abnormal lipid metabolism, improving oxidative stress and alleviating chronic inflammation. In each plate, there are many different types of targets, such as genes, proteins, receptors, pathways and so on. It can be concluded that, as a multi-target drug, BBR plays its role through various targets. Abbreviations: AMPK: AMP-activated protein kinase; PPAR: peroxisome proliferator activated receptors; ERK: extracellular regulated kinase; LDLR: low density lipoprotein receptor; IL-6: interleukin-6; IL-1: interleukin-1; CRP: C-reactive protein; TNF – α: tumor necrosis factor – α; SHBG: sex hormone binding globulin; AR: androgen receptor; StAR: steroidogenetic acute regulatory protein; AKR1C3: aldo-keto reductase 1C3; P450c17: p45017 α – hydroxylase; PTP1B: protein tyrosine phosphatase 1B; IRS1: insulin receptor substrate-1; MAPK: mitogen activated protein kinase; PEPCK: phosphoenolpyruvate carboxykinase; G-6-pase: glucose-6-phosphatase; SOD: superoxide dismutase; GSH-PX: glutathione peroxidase; MDA: malondialdehyde.

the total number of colonies in the gut of obese rats, as well as lower the abundance of butyrate producing bacteria in *Clostridium globosum* and *Clostridium flexnerum* subpopulation, thus reducing the degradation of polysaccharide in the diet and the intake of potential calories. The intestinal flora may affect the occurrence of PCOS and the disorder of glucose metabolism through the intestinal farnesoid X receptor (FXR) signal pathway [88]. However, another study [89] denied this claim. They found that the species diversity and amount of gut microbiota were directly cut down when BBR was used, but the condition of PCOS was not improved. It is not clear yet whether berberine can improve PCOS by interfering with intestinal microflora, and more research is needed.

6.3. BBR can reduce the risk of complications in PCOS patients

PCOS patients, especially those with IR, generally have the problems of long time of ovulation, high level of gonadotropin, high rate of cycle cancellation, high incidence of ovarian hyperstimulation syndrome (OHSS) and low clinical pregnancy rate. An Y et al [14]. believe that some effects of BBR, such as inducing ovulation, regulating menstruation, improving pregnancy rate and live birth rate, may be realized by acting on hypothalamus-pituitary-ovary axis. Their study confirmed that BBR can reduce the serum E2 level and the incidence of OHSS.

Besides, the incidence rate of endometrial cancer in patients with PCOS has been increasing year by year. Endometrium is continuously stimulated by estrogen, hyperinsulinemia and obesity, which are important factors contributing to the formation of endometrial cancer. BBR not only has plant anticancer ingredients, but also reduce the risk of cancer by significantly decreasing the expression of estrogen receptor α protein and reducing the biological function of estrogen in the local role of endometrium, so as to inhibit the sustained stimulation of estrogen on endometrium [90].

7. BBR in the treatment of PCOS

At present, the main treatment of PCOS is the combination of drugs and lifestyle adjustment including diet control, exercise and weight loss. Compound cyproterone acetate (CPA) is the first drug to adjust menstruation and reduce androgen. Insulin sensitizers such as metformin, thiazolidinedione and acarbose are needed if IR is combined. All those drugs require to be taken for a long time or even the entire life, in order to prevent and treat type 2 diabetes, hyperlipidemia, cardiovascular and cerebrovascular diseases and other complications. The significance of the treatment of the disease is not only to improve the pregnancy rate and pregnancy outcome, but also to reduce the risk and probability of long-term complications [91]. More and more researches [92] showed that the treatment of PCOS should focus on the interaction among all links and give full play to the advantages of natural drugs in multi-channel, multi-link and multi-target intervention in the pathological process. BBR has a wide range of pharmacological effects. It plays a role in the treatment of PCOS by relieving IR, reducing inflammation, improving lipid peroxidation, and regulating blood lipid, blood pressure, blood glucose and other aspects which involves multiple mechanisms of intervention, and gives BBR a high value for future research. Moreover, as a natural drug, BBR has a low adverse reaction rate. Only a few patients will have side-effects such as abdominal distension, constipation, mild stomachache, anorexia, *etc* [56]. In addition, BBR has a very small risk of hypoglycemia [93], which means it is safer.

BBR combined with other drugs is commonly used to treat PCOS. From a pharmacokinetic point of view, the bioavailability of BBR is low. Due to the malabsorption of BBR and its first pass effect in intestine, the oral absolute bioavailability of BBR in rats is less than 1% [94]. However, it has been reported that BBR is a high affinity substrate for some substances which are very important for drug transmembrane transport and metabolism, such as P-glycoprotein (P-gp), organic cation transporters and some cytochrome P450 isoenzymes. When BBR is combined with them, it can slow down the metabolism of other drugs, thus enhancing the efficacy [16]. In addition, the combination of BBR and absorption enhancer can also improve its own bioavailability. In the rats used BBR in combination, Mihwa Kwon [95] demonstrated increases in both initial plasma concentrations and area under the cure (AUC) of metformin, while decreases in systemic clearance and distribution volume of metformin. BBR tablets combined with metformin can reduce the

Table 1
BBR combined with other drugs in the treatment of PCOS.

Combination drug	Patient(s)	Effect of experimental group compared with control group											Reference
		BMI	HOMA (IR)	LH	T	FSH	LH/ FSH	SHBG	FBG	FINS	ovulation rate	pregnancy rate	
MET	56 women had PCOS	↓	↓	↓	↓	-	↓	/	-	↓	↑	/	[103]
		Conclusion: Combining BBR with Met can improve the condition of BMI and IR more effectively as well as the rate of ovulation condition											
	84 obese women had PCOS	↓	↓	↓	↓	-	↓	/	/	/	↑	/	[104]
		Conclusion: Combining BBR with Met can improve the obesity symptoms of obese women with PCOS, as well as reduce the hormone level and increase the ovulation rate											
CPA	100 women had PCOS	-	↓	/	↓	/	/	↑	↓	↓	/	/	[56]
		Conclusion: BBR intake improved some metabolic and hormonal disorders in a group of Chinese patients with polycystic ovary syndrome.											
	80 infertile women had PCOS	/	/	↓	↓	/	/	↑	↓	↓	↑	↑	[105]
		Conclusion: For patients with PCOS, CPA combined with BBR helps to regulate the fueling reproductive endocrine and glucose metabolism, promote the ovulation, increase pregnancy rate.											
	50 women had PCOS	↓	↓	↓	↓	-	/	/	↓	/	/	/	[106]
		Conclusion: BBR combined with CPA can effectively improve many pathophysiological changes, such as IR and high androgen levels in the treatment of PCOS.											
Yasmin	40 adolescents had PCOS	↓	-	-	-	-	-	/	↓	↓	/	/	[100]
		Conclusion: For adolescents with PCOS, BBR combined with Yasmin can improve the endocrine metabolism and clinical symptoms significantly.											
Letrozole	644 women had PCOS	/	/	/	/	/	/	/	/	/	/	-	[102]
		Conclusion: BBR combined with letrozole did not add fecundity in PCOS.											
	98 PCOS patients with IR	/	/	/	/	/	/	/	/	/	↑	/	[101]
		Conclusion: BBR combined with letrozole can promote ovulation in PCOS patients with IR.											
clomiphene	120 infertile women had PCOS	/	↓	↓	↓	↓	/	/	/	↓	↑	↑	[85]
		Conclusion: Combined BBR with clomiphene can improve endothelial function and endocrine, increase the rate of ovulation and pregnancy.											

Experimental group：BBR + Combination drug; Control group：Combination drug alone; ↓: the experimental group is significantly lower than the control group, and the difference is statistically significant ($P<0.05$); ↑: The experimental group is significantly higher than the control group, and the difference is statistically significant ($P<0.05$); -: The difference was not statistically significant; /: This item is not involved in the literature; MET: Metformin; CPA: compound cyproterone acetate;

inflammatory response, promote the secretion of insulin from insulin β cells, and improve the insulin sensitivity of cells, so as to achieve a variety of hypoglycemic effects [96–98] which is significantly improved compared with BBR or metformin alone. The results of An Y [14] study indicated that BBR and metformin can significantly increase the clinical pregnancy rate of PCOS patients, and BBR group also has a higher live birth rate. BBR and metformin have similar effects on improving HA and hyperinsulinemia of PCOS patients, both of which can improve the pregnancy rate of PCOS patients undergoing IVF treatment, and reduce the probability of OHSS.

The analysis of a study [99] showed that co-administration of BBR and metformin can not improve the effect of metformin on lowering BMI and HOMI-IR, but combining BBR with CPA can improve glucose metabolism and insulin sensitivity, suggesting that BBR can be used as insulin sensitizer.

In addition to the combination of BBR and metformin/CPA, one study [3] found that BBR combined with Pei Kun Wan can effectively improve the glucose and lipid metabolism of PCOS-IR patients, as well as their inflammatory response and sex hormone level, and the effect is better than metformin. BBR combined with yasmin is an effective treatment for adolescent PCOS, which can significantly improve the sex hormone, glucose metabolism, main clinical symptoms and signs [100]. BBR combined with letrozole has a synergistic effect on ovulation induction in PCOS patients with insulin resistance. The effect is better than metformin combined with letrozole. It can significantly improve the ovulation rate [101]. It is a safe and effective method, which is worthy of clinical application. But another study [102] held a different view. They believed that letrozole combined with BBR did not improve PCOS patients' fertility to some extent (Table 1).

It can be seen that more research is needed on whether BBR combined with other drugs can promote the treatment of PCOS.

8. Conclusion

BBR, as a multi-target, multi-path plant extract, can interfere with the development of PCOS and related pathological process from many aspects, with less adverse reactions than conventional drugs. It is mentioned in this review that BBR can alleviate IR, reduce the level of serum androgen, alleviate abnormal lipid metabolism and chronic inflammation. Summarizing the mechanism mentioned above, it is not difficult to find that alleviation of IR is the core mechanism of BBR in the treatment of PCOS. Other mechanisms can directly or indirectly affect IR. In addition, PPAR, MAPK and AMPK signaling are the key pathways for BBR to act on patients with PCOS. Due to the pharmacological effect of BBR, it is often used in combination with metformin, CPA and other drugs, in order to achieve better therapeutic results. Therefore, the mechanism and efficacy of BBR in the treatment of PCOS need further study, especially in the alleviation of IR.

9. Outstanding questions

This paper lists a number of studies that have demonstrated the therapeutic effect of BBR on PCOS. However, the mechanism of BBR in the treatment of PCOS is still unclear. At present, there are two opposite views on whether BBR can increase SHBG by alleviating insulin resistance to treat PCOS. In addition, how BBR works by increasing the production of adiponectin needs further study. At the same time, it is not clear whether BBR can improve PCOS by interfering with intestinal flora. Finally and most importantly, more research needs to focus on the therapeutic effect of BBR combined with other drugs, especially whether BBR can reduce adverse reactions and complications, so as to guide clinical medication more effectively.

Author contribution

The concept of the manuscript was devised by Zhang SW who also performed the overall literature searches. Both Zhang SW and Zhou J were in charge of writing. Table and Figures were designed by Zhang SW. Gober HJ, Leung WT and Wang L discussed the content of the article and gave suggestions.

Conflict of interest statement

We declare that we have no conflict of interest related to this work.

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