CLINICAL STUDY

A clinical study on the short-term effect of berberine in comparison to metformin on the metabolic characteristics of women with polycystic ovary syndrome

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Abstract

Objective: Polycystic ovary syndrome (PCOS) is a frequent reproductive and metabolic disorder associated with insulin resistance (IR). Berberine (BBR) is an isoquinoline derivative alkaloid extracted from Chinese medicinal herbs that has been used as an insulin sensitizer. BBR may have a potential therapeutic value for PCOS. The aim of this study was to evaluate the effects of BBR in comparison to metformin (MET) on the metabolic features of women with PCOS.
Design and methods: Eighty-nine subjects with PCOS and IR subjects were randomized into one of three treatment groups: BBR+compound cyproterone acetate (CPA; $n=31$), MET+CPA ($n=30$), and placebo+CPA ($n=28$) for 3 months. Clinical characteristics of the women and metabolic and hormonal parameters were assessed before and after the period of treatment.
Results: Treatment with BBR in comparison to MET showed decrease in waist circumference and waist-to-hip ratio (WHR; $P<0.01$), total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDLC; $P<0.05$) as well as increase in high-density lipoprotein cholesterol (HDLC) and sex hormone-binding globulin (SHBG; $P<0.05$). Similarly, treatment with BBR in comparison to placebo showed decrease in WHR, fasting plasma glucose, fasting insulin, homeostasis model assessment for IR, area under the curve of insulin, TC, LDLC, and TG ($P<0.05$) as well as increase in HDLC and SHBG ($P<0.01$).
Conclusions: Intake of BBR improved some of the metabolic and hormonal derangements in a group of treated Chinese women with PCOS. Main effects could be related to the changes in body composition in obesity and dyslipidemia. Further controlled studies are needed for the assessment of the potential favorable metabolic effects of BBR in women with PCOS.

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Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine and metabolic disorder afflicting 3–8% of women of reproductive age. PCOS is characterized by chronic anovulation, hyperandrogenism, and/or the presence of polycystic ovary morphology (1). A wide variety of risk factors have been investigated in relation to PCOS, including obesity, glucose intolerance, and dyslipidemia, which lead to a significantly increased risk for long-term type 2 diabetes mellitus (T2DM) and cardiovascular disease (2–5). Insulin resistance (IR) is known to play a critical role in the pathophysiology of PCOS (6, 7). The administration of insulin sensitizer metformin (MET) is recognized as a successful treatment for many metabolic and reproductive dysregulations characteristic of women with PCOS (8–13).

Berberine (BBR, $[C_{20}H_{18}NO_4]$) is a type of isoquinoline derivative alkaloid extracted from Chinese medicinal herbs, such as Coptidis Rhizoma (Huanglian), Cortex Phellodendri (Huangbai), and Hydrastis Canadensis (goldenseal). In China, BBR is a non-prescription oral drug for the treatment of gastrointestinal infections and diabetes. BBR possesses a wide range of biochemical and pharmacological activities, including anti-obesity and anti-dyslipidemia (14–16). A large body of evidence has demonstrated that BBR is an effective insulin sensitizer and has a comparable activity to MET in reducing IR (15, 17–19). However, before we can draw conclusions regarding the pharmacological

properties of BBR and their applicability to the pathophysiological changes observed in PCOS, trials examining the therapeutic value of BBR in patients with PCOS are required.

This study aimed to assess whether use of BBR, considered to be an insulin sensing agent, is effective in the treatment of endocrine characteristics of PCOS and to compare these effects with MET therapy. The outcome measures in this study included change in anthropometric measures and hormonal and metabolic indices in a group of insulin-resistant Chinese women with PCOS.

Subjects and methods

Subjects

A total of 100 subjects with PCOS and insulin resistance (IR) were included in this study. They were recruited from the Clinical Centre of Reproductive Medicine of the First Affiliated Hospital of Harbin Medical University (Harbin, China). The diagnostic criteria for PCOS followed the 2003 Rotterdam ESHRE/ASRM criteria. Oligomenorrhea (interval between menstrual periods ≥35 days) or amenorrhea (absence of vaginal bleeding for at least 6 months) [20] and clinical (a Ferriman–Gallwey score ≥6) [21] and/or biochemical hyperandrogenism (total testosterone (TT) ≥58 ng/dl (2 nmol/l)) were used to assess PCOS. Normal range of TT in healthy Chinese women is no more than 2 nmol/l [22]. The phenotype of polycystic ovaries was detected by vaginal ultrasound examination presenting 12 follicles or more in one or both ovaries and/or increased ovarian volume (>10 ml) [23]. All subjects fulfilled at least two of the three diagnostic criteria. IR was assessed by homeostasis model assessment for IR (HOMA-IR) ≥3.8 [24] or fasting glucose insulin ratio (FGIR) ≤4.5 [25].

Exclusion conditions included the following systemic and endocrine disorders: late-onset congenital adrenal hyperplasia, Cushing's syndrome, thyroid dysfunction, hyperprolactinemia, diabetes mellitus, coronary artery disease, and spontaneous abortion. Furthermore, subjects accepting treatment with medications known to alter insulin hemodynamics, ovulation induction, anti-obesity, or oral contraceptives (OCs) within 3 months were excluded from the study. All subjects were nonsmokers, and none reported chronic alcohol consumption.

The study was conducted according to the Helsinki Declaration on human experimentation. The study protocol was approved by the ethics committee of the First Affiliated Hospital of Harbin Medical University. The registration number of the trial is 200907. The purpose, procedure, and potential risks of the study were carefully explained to each subject, and written consent was obtained before beginning the study.

Methods

All subjects received lifestyle modification and antiandrogen compound cyproterone acetate (CPA) in the form of combined pill with ethinyl estradiol, randomly assigned to the BBR, MET, or placebo groups for a 3-month treatment course. Randomization was based on a computer-generated code in blocks of six. A copy of the code was stored in a sealed envelope by personnel not involved in the trial.

All subjects received advice from a nutritionist on nutrition and exercise. They were instructed to limit fat and carbohydrate intake and improve dietary behavior without the application of a calorie-restricted diet program. Exercise was also recommended to include 30 min/day of moderate to intense activity and was not monitored. CPA (Diane 35, Bayer Schering Pharma) containing 35 μg ethinyl estradiol and 2.0 mg cyproterone acetate were also taken in a cyclic fashion. BBR (BBR hydrochloride, Northeast General Pharmaceutical Factory, Harbin, China) was administered at a dosage of 3×500 mg daily, MET (Bristol-Myers Squibb Company, Shanghai, China) was administered at a dosage of 3×500 mg daily, except for the first week of treatment when 500 mg was given only twice a day to reduce the incidence and severity of gastrointestinal side effects. Placebo (provided by pharmaceutical preparation section) was administered as one tablet twice a day.

Clinical and endocrine examination

Clinical assessments included anthropometric measurements (height (m), weight (kg), body mass index (BMI), and waist-to-hip ratio (WHR)). BMI was calculated as weight divided by height squared. WHR was defined as the ratio between the circumference of waist and hip. Blood pressure was measured in the seated position in the right arm after a 30 min rest period and averaged over three measurements.

In the baseline study, vaginal bleeding in the absence of spontaneous bleeding was induced by progesterone withdrawal. After a 12 h overnight fast, a venous blood sample was obtained in the morning between 0800 and 0900 h during the early follicular phase (days 2–3). In all subjects, circulating concentrations of the reproductive hormones TT and sex hormone-binding globulin (SHBG) were measured. The free androgen index (FAI) = (testosterone (nmol/l)/SHBG (nmol/l)) $\times$ 100 [26] was also calculated. Other parameters included fasting plasma glucose (FPG) and insulin (FIN), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDLC), and low-density lipoprotein cholesterol (LDLC). Immediately after obtaining the basal blood sample, a 2 h, 75 g oral glucose tolerance test (OGTT) was performed, and blood samples were obtained after 30, 60, and 120 min to measure serum glucose and insulin concentrations. The degree of IR was estimated by a number of different methods, including calculated

FGIR, HOMA-IR, and area under the insulin curve (AUC_{INS}). Impaired glucose tolerance (IGT) was defined as fasting glucose > 5.6 mmol/l and/or 2–h glucose > 7.8 mmol/l (27).

FGIR = FPG (mg/dl)/FIN (mIU/ml) (24).

HOMA-IR = FIN (mIU/ml) × FPG (mmol/l)/22.5 (23).

AUC_{INS} = insulin $0' \times 0.25$ + insulin $30' \times 0.5$ + insulin $60' \times 0.75$ + insulin $120' \times 0.5$ (28).

After 3 months of treatment, each subject underwent the same procedures as described above.

Assays

Serum concentrations of TT, SHBG, and insulin were measured using the electro-chemiluminescent immuno-assay method by auto-analyzer (Abbott I2000). Plasma glucose, TC, TG, HDLC, and LDLC levels were determined using the electro-chemiluminescent immunoassay method by auto-analyzer (Abbott V51).

Statistical analysis

SPSS 13.0 (SPSS Inc., Chicago, IL, USA) was used for the data analysis. A repeated measures ANOVA was used to estimate the statistical difference between treatment groups, including pre- and post-treatment and within one group between pre- and post-treatment. Logarithmic transformations of FGIR, HOMA-IR, and AUC_{INS} were applied before ANOVA to ensure homogeneity of variances. Data are presented as mean ± s.d. with a value of $P < 0.05$ considered as statistically significant.

Results

All subjects were reproductive-aged Chinese females living in the Heilongjiang Province. Complete data from 89 of the 100 subjects were analyzed. One subject from the BBR group, two from the MET group, and two from the placebo group were lost to follow-up after randomization without any visits. Two subjects from the BBR group and one from the MET group left for personal reasons. Two subjects from the MET group and one from the placebo group left because of travel difficulties. The final number of subjects in the three groups were similar (BBR 30, MET 31, and placebo 28). Nine subjects who received MET complained of transient abdominal discomfort (nausea, vomiting, mild diarrhea, and flatulence) and three subjects who received BBR complained of a bitter taste in their mouth.

Clinical characteristics of the studied subjects

At baseline, there were no significant differences in any parameter among the groups (Table 1). After 3 months of treatment, body weight and BMI decreased significantly in all three groups; however, compared to placebo, no significant differences in body weight and BMI were observed in the BBR ($P=0.928$, $P=0.161$) and the MET groups ($P=0.146$, $P=0.975$). BBR and MET had no additive effect on decreasing weight and BMI. Waist circumference (WC) and WHR also decreased in all of the groups after 3 months of treatment. However, a greater reduction in WC was observed in the BBR group (88.38 ± 5.84 vs 80.22 ± 5.17) than in

Table 1 Clinical characteristics and metabolic parameters of all subjects before and after treatment.

Parameters	BBR+CA (n=31; age=25.74±2.66)		MET+CA (n=30; age=26.03±2.82)		PL+CA (n=28; age=26.75±2.62)	
	Pre	Post	Pre	Post	Pre	Post
Weight (kg)	65.11±4.06	60.06±3.61	62.98±5.21	58.58±4.66	65.13±5.10	60.16±3.95
WC (cm)	88.38±5.84	80.22±5.17	89.12±7.06	85.03±6.53[‖]	85.23±6.18	80.75±5.28[¶]
BMI (kg/m²)	25.57±1.60	23.64±1.45	24.74±1.85	23.03±1.72	24.91±1.66	23.01±1.27
WHR	0.89±0.03	0.82±0.03[§]	0.9±0.04	0.87±0.03	0.89±0.05	0.87±0.44[¶]
FPG (mmol/l)	4.97±0.67	4.36±0.53[§]	5.10±0.69	4.61±0.55	5.07±0.67	4.63±0.73
FIN (mU/l)	19.77±5.80	13.07±2.45[§]	21.94±12.43	13.81±4.92[‖]	21.04±10.71	17.80±4.60
OGTT (nmol/l)	7.82±0.58	6.93±0.93	7.76±0.46	7.06±0.92	7.71±0.42	7.53±0.53
HOMA-IR	4.38±1.49	2.55±0.65[§]	4.92±2.61	2.82±1.02[‖]	4.78±2.57	3.76±0.92
FGIR	4.81±1.19	6.19±1.25[§]	4.85±1.50	6.60±2.10[‖]	4.82±1.23	5.09±1.21
AUC_{INS}	231.55±61.72	121.66±14.72[§]	258.84±90.59	122.43±11.80[‖]	238.69±59.97	134.74±16.39
TC (mmol/l)	5.85±0.39	4.65±0.52[§]	5.70±0.51	5.01±0.42	5.71±0.47	5.03±0.39[‡]
TG (mmol/l)	2.35±0.27	1.95±0.24[*]	2.26±0.25	2.09±0.22	2.30±0.24	2.10±0.23[‡]
LDLC (mmol/l)	4.22±0.56	3.62±0.60[§]	4.25±0.51	3.95±0.48	4.28±0.52	4.05±0.51[‡]
HDLC (mmol/l)	1.11±0.12	1.24±0.09[§]	1.13±0.13	1.19±0.10[†]	1.11±0.12	1.13±0.11[‡]
TT (nmol/l)	1.89±0.14	1.47±0.22[*]	1.88±0.13	1.53±0.20	1.85±0.12	1.59±0.17
SHBG (nmol/l)	25.37±4.31	58.70±11.03[§]	24.20±3.52	51.98±9.66[‖]	24.19±4.01	45.01±8.32[¶]
FAI (%)	7.69±1.55	2.59±1.12[§]	7.92±1.22	3.16±0.98[†]	7.81±1.15	3.77±1.38

*$P<0.05$, BBR vs placebo; [†]$P<0.05$, MET vs placebo; [‡]$P<0.05$, BBR vs MET; [§]$P<0.01$, BBR vs placebo; [‖]$P<0.01$, MET vs placebo; [¶]$P<0.01$, BBR vs MET. CA, cyproterone acetate; PL, placebo; CPA, compound cyproterone acetate; WC, waist circumference; BMI, body mass index; WHR, waist-to-hip ratio; FPG, fasting glucose; FIN, fasting insulin; OGTT, oral glucose tolerance test; HOMA-IR, homeostasis model assessment for insulin resistance; AUC_{INS}, area under the curve of insulin; TC, total cholesterol; TG, triglycerides; LDLC, low-density lipoprotein cholesterol; HDLC, high-density lipoprotein cholesterol; TT, total testosterone; SHBG, sex hormone-binding globulin; FAI, free androgen index.

the MET group (89.12 ± 7.06 vs 85.03 ± 6.53; $P = 0.001$). WHR decreased from 0.89 ± 0.03 to 0.82 ± 0.04 in the BBR group, a significantly greater reduction than that observed in the MET (0.9 ± 0.04 vs 0.87 ± 0.03; $P < 0.001$) and the placebo groups (0.89 ± 0.05 vs 0.87 ± 0.44; $P < 0.001$) (Table 1).

Carbohydrate metabolic parameter of the studied subjects

After 3 months of treatment, the placebo group, FIN, HOMA-IR, and AUC_{INS} showed a significant reduction compared with the baseline (Table 1). Other markers of IR, such as FGIR and FPG, remained unchanged; however, compared with the subjects who received placebo, subjects receiving BBR or MET had a significant improvement in insulin sensitivity. FPG decreased from 4.97 ± 0.67 to 4.36 ± 0.53 with BBR ($P = 0.005$). FIN significantly decreased after treatment with BBR (19.77 ± 5.80 vs 13.07 ± 2.45) or MET (21.94 ± 12.43 vs 13.81 ± 4.92). Moreover, a significant decrease in HOMA-IR and AUC_{INS} was also detected in the BBR and MET groups. Conversely, FGIR was significantly increased after treatment with BBR (4.81 ± 1.19 vs 6.19 ± 1.25; $P = 0.004$) or MET (4.85 ± 1.50 vs 6.60 ± 2.10; $P = 0.001$). However, no significant variation in FPG, FIN, FGIR, HOMA-IR, and AUC_{INS} was noted between the BBR and MET groups (Table 1).

Lipid profile parameters of the studied subjects

After 3 months of treatment, reductions in TC and TG were observed in all of the groups. In the placebo group, the level of LDLC showed a decreasing trend but was not statistically significant, and the level of HDLC remained unchanged. The BBR group demonstrated a significant decrease in TG from 2.35 ± 0.27 to 1.95 ± 0.24, TC from 5.58 ± 0.39 to 4.65 ± 0.52, and LDLC from 4.22 ± 0.56 to 3.62 ± 0.60. Compared with the MET group, the differences of TG, TC, and LDLC were statistically significant ($P = 0.034$, $P = 0.012$, $P = 0.016$). Additionally, BBR therapy caused a significant increase in the plasma level of HDLC from 1.11 ± 0.12 to 1.24 ± 0.09, whereas MET treatment only increased HDLC from 1.13 ± 0.13 to 1.19 ± 0.10 ($P = 0.039$) (Table 1).

Reproductive hormone levels of the studied subjects

TT and FAI decreased, whereas SHBG increased significantly in all of the groups after 3 months of treatment. The level of SHBG increased significantly in the BBR group (25.37 ± 4.31 vs 58.70 ± 11.03) compared with the MET ($P = 0.008$) and placebo groups ($P = 0.006$). BBR treatment resulted in a significant decline in TT from 1.89 ± 0.14 to 1.47 ± 0.22 and a corresponding decline of FAI from 7.69 ± 1.55 to 2.59 ± 1.12, which were significantly different from the changes in the placebo group ($P = 0.017$, $P = 0.0002$). However, the changes in TT and FAI after BBR treatment were not significantly different from the changes observed following MET treatment ($P = 0.15$, $P = 0.06$) (Table 1).

Discussion

In recent years, the recommended management strategy for long-term treatment of PCOS includes lifestyle modification, insulin sensitizers, and OC. This comprehensive intervention protocol results in better regularity of menses and fertility potential (10, 29, 30). In this study, OC and lifestyle modification remained the first-line therapy choice and every subject received CPA in the form of a combined pill during the 3-month treatment period. Furthermore, BBR, MET, and placebo were administered randomly in order to evaluate the effects of BBR in comparison to MET in a group of women with PCOS. However, this study had two limitations: small subgroups were studied and the study population was under the treatment of OC.

Compared with the baseline, significant improvement in biochemical indices of hyperandrogenemia like TT and FAI, were observed in all of the groups after 3 months of treatment. Our data suggest that both BBR and MET have similar effects on reduction of concentrations of androgens, which are in agreement with the results of other studies analyzing effects of MET in PCOS (9, 31).

MET as the most commonly used insulin sensitizer exerts favorable metabolic outcomes in women with PCOS, such as decrease in insulin and androgen concentrations, normalization of menstrual cycles, and improvement ovulation rates (10, 32, 33). Although, our 3-month treatment period led to decrease in concentrations of insulin and androgens as it was reported elsewhere (11, 12, 32, 33), we could not confirm significant effect of MET on weight change and lipid profile as it was shown by others (11, 12, 34). Three months may be too short a time to demonstrate the effect of MET on metabolic abnormalities sufficiently. Furthermore, MET is associated with a higher incidence of nausea, vomiting, and other gastrointestinal disturbances.

BBR, as the main constituent of traditional Chinese medicine, displays good potential in the prevention and treatment of metabolic disorders, including weight control, cholesterol reduction, and antilipogenic and hypoglycemic effects (35). Several trials have reported that BBR treatment can not only reduce body weight and the ratio of white adipose tissue to body weight (7, 14) but also increase energy expenditure and the consumption of lipid metabolites as the primary energy source in obese animals (36). In our study, body weight and BMI decreased in all three groups after treatment. Compared with the placebo group, subjects treated with

BBR showed no significant differences in body weight and BMI. Therefore, losing weight may be attributed to lifestyle modifications rather than BBR therapy, which conflicts with previous results that demonstrated BBR's anti-obesity activity (14, 37). It is possible that the treatment period did not last long enough for the effect of BBR on body weight to be observed. However, WC and WHR in the BBR group decreased significantly compared with the MET and placebo groups, which supports the notion of BBR-induced adipose tissue redistribution and ameliorated central fat distribution in PCOS, with significantly decreased WC and WHR of the subjects in the absence of weight changes.

BBR has been reported to exert hypoglycemic effect in either animal models or on humans and has a comparable activity to MET as an insulin sensitizer. Moreover, BBR improves IGT and reduces blood glucose without increased insulin release and synthesis (38). A growing body of evidence suggests that BBR improves insulin sensitivity and stimulates glucose uptake via activation of the AMP-activated protein kinase pathway (14, 39–41), which may be the target for BBR-induced regulation of glucose and lipid metabolism. Our study confirmed that administration of BBR (0.5 g t.i.d.) was able to reduce FBG, FIN, HOMA-IR, and AUC_{INS} in patients with PCOS, and the outcomes were consistent with previous reports. However, no significant variations in glycemic parameters were noted between the BBR and the MET groups. It could be demonstrated that BBR and MET exerted comparable effects on improving glucose metabolism in PCOS.

In addition to the hypoglycemic action, a beneficial effect of BBR on lipid metabolism was also observed in animals and human subjects (7, 14, 15, 37, 42). The mechanism of BBR-moderated lipid metabolism is related to upregulation of LDL receptor in both mRNA and protein in liver (7). In this study, the effect of BBR on lipid metabolic parameters was demonstrated by the significant decrease in serum concentrations of TC, TG, and LDLC and the HDLC increase after 3 months of treatment. Although BBR and MET both improved glycemic parameters, their effects on lipid metabolism were different. Compared with BBR, MET was less effective on the lipid parameters. BBR-induced alleviation of lipid dysregulation surpassed those obtained by MET in this study.

Until now, there have been few reports concerning the effect of BBR use on reproductive hormones. A recent study with dexamethasone-induced IR on theca cells showed an excessive testosterone production and that was effectively antagonized by BBR (43). In our study, level of testosterone and FAI significantly decreased in the group treated with BBR in comparison to the placebo group. Although the mechanism of BBR-reducing hyperandrogenism remains to be clarified, it may be partially related to the remarkable effect of BBR on amelioration of IR.

With regard to safety, BBR is not considered toxic at present doses used in clinical situations (37). Recent studies reported that by treatment with BBR (0.5 g t.i.d.) none of the subjects were observed to have marked changes in renal and hepatic function. The pharmacokinetics of BBR studied in rats also suggests that blood clearance of BBR is very quick and that its biotransformation in the liver is rapid (44). The major side effects of BBR can result from overdose, including diarrhea, constipation, flatulence, and abdominal pain in rare cases (7). Subjects in this clinical study tolerated BBR well and none had mild gastrointestinal discomfort. No data are available regarding the safety of BBR in long-term use in young women and on its safety in early pregnancy.

In conclusion, we showed that BBR in comparison to MET showed similar metabolic effects presumably on amelioration of insulin sensitivity and reduction of hyperandrogenemia. BBR also appeared to have a greater effect on the changes in body composition and dyslipidemia. However, the underlying mechanisms of its action remain to be clarified in longer term clinical trials that are required to evaluate therapeutic efficacy and safety of BBR in women with PCOS.

Declaration of interest

Funding

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