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Original article

Berberine improves reproductive features in obese Caucasian women with polycystic ovary syndrome independently of changes of insulin sensitivity

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ABSTRACT

Background and aims: Berberine (BBR) is an isoquinoline derivative alkaloid isolated from Rhizoma Coptidis traditionally used as anti diarrheic and, more recently, as hypolipidemic and insulin sensitizer agent. Thus, BBR could represent a potential therapeutic option for patients with polycystic ovary syndrome (PCOS). The aim of this study was to evaluate the clinical, metabolic and hormonal effects of BBR in PCOS women.

Methods: Fifty oligoamenorrheic PCOS obese women and 50 age and Body Mass Index (BMI) matched healthy controls were enrolled. PCOS women received BBR treatment (500 mg, 2 times daily) for 6 months. Clinical and biochemical parameters were assessed before and after the treatment period.

Results: Total testosterone ($p < 0.01$), free androgen index ($p < 0.01$), androstenedione ($p < 0.01$), sex hormone binding globulin ($p < 0.01$), progesterone ($p < 0.01$), total cholesterol ($p = 0.01$), low density lipoprotein cholesterol ($p < 0.01$), triglycerides ($p < 0.01$), area under the curve of insulin ($p < 0.01$), menses frequency ($p < 0.01$) and Waist Circumference ($p = 0.04$) significantly ($p < 0.05$) improved after BBR treatment. No correlation was found between variations of insulin sensitivity and hormonal changes.

Conclusions: BBR improves clinical, metabolic and reproductive features in PCOS women. Its mechanism of actions need to be elucidated in further studies.

1. Introduction

Berberine (BBR) is an isoquinoline alkaloid extracted from the dry roots of coptidis rhizome and used in traditional Chinese medicine for its anti-diarrheal activity.[1] However, in the last two decades, a number of studies have clearly demonstrated beneficial metabolic effects including improved insulin resistance, visceral adiposity and atherogenic dyslipidemia. The anti-hyperglycemic effect of BBR has been confirmed by a recent meta-analysis carried on both drug naive and pharmacologically treated patients with type 2 diabetes.[2] BBR lowers glucose levels through multiple mechanisms though it seems to mainly reside in its pharmacological activity of insulin sensitizer. Recently, it has been shown that administration of BBR increased glucose infusion rate during hyperinsulinemic euglycemic

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clamp in high fat-fed rats, confirming its ability to improve insulin resistance (IR).[3] In addition, BBR is an effective lipid-lowering drug as demonstrated by a clinical study in which the administration of BBR in hypercholesterolemic patients for 3 months reduced low density lipoprotein cholesterol (LDL) by 25%.[4]

Due to its positive metabolic properties, BBR might act as a valuable medication in IR states such as metabolic syndrome and polycystic ovary syndrome (PCOS). In particular, PCOS, the most common endocrine disorder in the reproductive-age women, is characterized by several risk factors for type 2 diabetes mellitus and cardiovascular diseases such as abdominal obesity, glucose intolerance and dyslipidemia.[5,6]

Based on this background, a recent study investigated the role of BBR compared to metformin treatment in women with PCOS.[7] The main finding of this study[7] was that intake of BBR improved some metabolic and hormonal derangements in women with PCOS. However, this study[7] shows some limitations and bias, such as the lack of a pure evaluation of the effect of BBR, in fact it was associated with ciproterone acetate, and the short length of the study, i.e. 3 months.[7] Therefore, the still opened question remains whether BBR is able by itself to improve metabolic and hormonal derangements in women with PCOS.

Thus, the aim of our study was to investigate whether the use of BBR may improve IR and hormonal profile in obese women with PCOS. The secondary outcome was to assess whether metabolic effects were related to the changes of any hormonal parameters.

2. Materials and methods

2.1. Study population

Fifty PCOS obese women and 50 age and body mass index (BMI) matched premenopausal healthy controls were enrolled in two Italian centers: Salerno University Hospital – ART Unit and "CMSO" Antidiabetic Center in Salerno after obtaining written consent. The protocol was approved by Local Ethical Committee. Diagnosis of PCOS was based on the Rotterdam criteria that required two out of three of the following criteria to fulfill the diagnosis: oligo-and/or anovulation; clinical and/or biochemical signs of hyperandrogenism and polycystic ovaries (PCO) by ultrasound.[8] Oligo- and/or anovulation were defined by the presence of oligomenorrhea or amenorrhea.[9] Oligomenorrhea was defined as infrequent or irregular menses while amenorrhea occurred if a woman missed three or more menstruation in a row. Hyperandrogenism was defined by the clinical presence of hirsutism (Ferriman–Gallwey score $\geq$ 8),[10] and/or elevated androgen levels (testosterone $>$ 0.60 ng/ml and free androgen index $>$ 7%)[11].

Transvaginal ultrasonographic examinations were performed by the same experienced operator during the early follicular phase (2nd–3rd day) of a spontaneous or progesterone (P)-induced bleedings. Ovarian dimension and morphology were noted bilaterally in each subject. In particular, ovarian dimensions were obtained by measuring the main three diameters and applying the ellipsoid formula, and ovarian morphology was defined as PCO or not PCO according to published criteria.[12]

Other common causes of hyperandrogenism and/or anovulation (hyperprolactinemia, congenital adrenal hyperplasia, Cushing syndrome, and virilising or adrenal tumors) were excluded. All women reported no use of any medication during the last 2 months that could interfere with endocrine and metabolic parameters.

Throughout the study, no lifestyle modification was implemented; indeed, participants were instructed to follow their usual diet and physical activity. Each patient was also adviced to use physical barrier contraception throughout the study.

PCOS women received BBR (Berberol™, PharmExtracta, Pontenure, Italy, an oral tablet containing 588 mg of *Berberis aristata* extract titered as 85% berberine and 105 mg of *Silybum marianum* extract titered as >60% flavonolignans) twice daily (before breakfast and dinner) for 6 months.

All participants were instructed to record in a personal daily diary the number of skipped tablets (specifying the reason: missed, fear for side effects, other), the onset of any adverse events (specifying their characteristics: severity, duration, and possible cause-effect relationship with drug administration), the characteristics of their menstrual cycles (length and quantity), and any changes in diet, physical activity or weight. During the study, no monitoring of ovulation was performed by ultrasonography. However, ovulation was confirmed by a plasma progesterone assay (>10 ng/ml, SI: 32 nmol/l) performed 7 days before the expected menses in patients who reported a regular vaginal bleeding the month before. Irregular periods were defined when cycle length was higher than 35 days.[12]

For all subjects, BMI was calculated as weight (kg)/height (m^2). Waist circumference (WC) was measured in a standing position midway between the lower costal margin and the iliac crest. Hip circumference was measured in a standing position at the maximum circumference over the buttocks and the waist-to-hip ratio (WHR) calculated.

During the early follicular phase of the menstrual cycles (spontaneous or P-induced cycles) and after fasting overnight for 10–12 h, blood samples were collected for the following assays: follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone, androstenedione, 17-hydroxyprogesterone, sex hormone binding globulin (SHBG), dehydroepiandrosterone sulfate (DHEAS), triglycerides, total cholesterol, and high density lipoprotein (HDL) and LDL cholesterol.

Free androgen index (FAI) was calculated as the ratio between total testosterone and SHBG as previously reported.[13] The menses frequency was calculated as the number of observed menses/number of expected cycles ratio. The cumulative rate of normal cycling women was calculated as total numbers of women normal cycling (at least 2 consecutive menstrual cycles with P levels diagnostic for ovulation) after 6 months of BBR administration.

2.2. Biochemical assays

Testosterone, androstenedione, DHEA-S, and estradiol were measured by specific radio-immuno assay (RIA) as previously described.[13] SHBG was measured using an immunoradiometric assay. Blood glucose levels were determined by the glucose oxidase method, and serum insulin was measured by a solid-phase chemiluminescent enzyme immunoassay.

The serum total cholesterol, HDL, LDL, and triglycerides levels were measured with an autoanalyzer (Monarch 1000; Instrumentation Laboratory, Milan, Italy) using commercial kits (IL TEST; Instrumentation Laboratory).

All patients underwent the 75-gr oral glucose tolerance test (OGTT). Plasma glucose and insulin concentrations were measured at 0, +30, +60, +90, and +120 min after glucose load. IR was assessed by homeostasis model assessment of insulin resistance (HOMA-IR).[14]

Overall, intra- and inter-assay coefficients of variation (CVs) were less than 10%.

2.3. Statistics

Statistical analysis was carried out using SPSS 9.0. Data are expressed as mean ± standard deviation (SD). Depending on the distribution of the data, the Student's *t*-test for independent