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Original Research Article

Berberine regulates endocrine function in mice with polycystic ovary syndrome through PI3K/Akt/GSK-3β insulin signaling pathway

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Abstract

Purpose: *To study the influence of berberine on endocrine status in mice with polycystic ovary syndrome (PCOS), and the underlying mechanism of action.*
Methods: *A total of 80 mice were used in this research. Sixteen mice were randomly selected to serve as control. The remaining 64 mice were subcutaneously given dehydroepiandrosterone (DHEA) injection to establish a mouse model of PCOS. The PCOS mice were randomly divided into model group, and low-dose-, medium-dose and high-dose berberine groups. Oral glucose tolerance test (OGTT) and expression levels of PI3K/Akt/GSK-3 signaling pathway related proteins (PI3K 85, Akt2, p-GSK-3 Tyr216, p-GSK-3β Ser9, and GSK-3) were evaluated.*
Results: *At 60 and 120 min, OGTT blood glucose level of model group was significantly higher than that of blank control group, but it was significantly lower in the berberine dose groups than in model group ($p < 0.05$). There were significantly higher protein expression levels of pi3k85, AKT2 and p-GSK-3β tyr116 in berberine dose groups than in model mice, but the protein levels of p-GSK-3β ser9 in berberine dose groups were significantly lower than that in model mice.*
Conclusion: *Berberine improved endocrine function in PCOS mice through a mechanism involving regulation of the key proteins of PI3K/Akt/GSK-3 β insulin signaling pathway. Thus, berberine may potentially play a similar role in humans with PCOS functions. However, clinical trials need to be carried out first.*

Keywords: *Polycystic ovary syndrome, PCOS, Berberine, PI3K, Akt, GSK-3β*

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INTRODUCTION

The etiology and pathology of PCOS are extremely complex. The disease is highly prevalent in women of reproductive age, and it usually presents with reproductive, metabolic and endocrine abnormalities [1]. Research has confirmed that the etiology of PCOS is linked to the hypothalamus, insulin resistance (IR) and lipid metabolism [2]. Moreover, although PCOS has become rampant, not much is known about its pathogenesis. Moreover, there is need for further improvement in the diagnostic criteria of the disease. Therefore, it is necessary to develop

a method capable of enhancing clinical treatment efficacy and minimizing the risk of long-term complications. Currently, western drugs are used for treating PCOS patients of reproductive age, but these drugs have certain toxic side effects on the liver and kidney [3]. In the past 20 years, several studies have been done in the field of traditional Chinese and western medicines.

Berberine is extracted from *Coptis chinensis* and *Phellodendron phellodendron,* and it exerts bactericidal and antibacterial effects. Studies have confirmed that berberine exerts an insulin sensitization effect and also regulates glucose and lipid metabolism [4]. Phosphatidylinositol-3 kinase (PI3K) and threonine kinase (Akt) affect the activation state of many downstream effector molecules, and play key regulatory roles in cell apoptosis and proliferation [5].

Glycogen synthase kinase-3β (GSK-3β) regulates glycogen metabolism, and it is a downstream gene of PI3K/Akt route associated with tumor invasion and migration [6]. However, there are limited studies on the correlation between berberine and PI3K/Akt/GSK-3β pathway. Therefore, this research was focused on investigating the influence of berberine on endocrine function of mice with PCOS, and involvement of PI3K/Akt/GSK-3β insulin signal route in the process.

EXPERIMENTAL

Animals

Eighty (80) Wistar mice weighing 18 - 22 g were purchased from Wuhan Yihong Technology Co. Ltd. [batch number: SCXK (E) 2020-0001]. All mice were reared in cages, with 5 mice in each cage, and then placed in SPF animal house with indoor temperature of 23 ± 2 ^{o}C and humidity of 55 ± 5 %, in an environment with 12-h daylight. The mice were given *ad libitum* access to feed and water, and the cages were cleaned once every 3 to 4 days.

Main reagents and equipment

DHEA was purchased from Shanghai Guangrui Biotechnology Co. Ltd. Berberine was obtained from Wuhan Bid-Winning Technology Co. Ltd. Physiological saline (0.9 % NACI) was product of Shanghai Jingke Chemical Technol. Co. Ltd; ELISA kits were products of Beijing Shanben Biotechnology Co. Ltd, while 0.50 % glucose solution was purchased from Beijing Baiaolaibo Technology Co. Ltd, and BCA protein quantitative kit was purchased from Shenyang Wanshi Biotech. Co. Ltd., while ECL luminescent solution was product of Guangzhou Jinde Biotechnology Co. Ltd. Cell lysis buffer was purchased from Shanghai Fantai Biotechnology Co. Ltd, while Akt2 and GSK-3β antibodies were bought from Merck Life Sciences.

Optical microscope was supplied by Dingzhou Baikesi Biological Tech. Co. Ltd. Homogenizer was bought from Shanghai Daluo Scientific Instrument Co. Ltd. Low-temperature-centrifuge was product of Sichuan Shuke Instrument Co. Ltd. Blood glucose meter was obtained from Shanghai Xinfan Biotechnology Co. Ltd. Gel-forming system was bought from Beijing Ganming Gene Technology Co. Ltd, while electrophoresis apparatus was purchased from Jiangsu Bomeida Life Science Co. Ltd.

Study design

Sixteen (16) mice were randomly selected as blank control group. The remaining 64 mice were subcutaneously injected with 0.1mL DHEA solution at a dose of 0.6 mg/kg for 20 days, to establish a mouse model of PCOS, while control mice received injection of an equivalent volume of injection water for 20 days. Thereafter, the PCOS mice were assigned to into model, and low-, medium- and high-dose berberine groups. Mice in the berberine groups were intragastrically given berberine at doses of 10, 20 and 40 mg/kg/day, respectively, while mice in model group were intragastrically given 0.9 % NACI.

On day 21, mice in each group were fasted for 12 h without water. Blood samples were collected from the orbital venous plexus and centrifuged to obtain sera which were kept frozen at -80 ℃. Serum testosterone (T) and 17 hydroxyprogesterone (17-OHP) levels were determined using ELISA and radioimmunoassay, respectively. The levels of estradiol (E2) and progesterone (P) were determined with chemiluminescence.

After fasting the mice for 12 h, tail tip blood was collected from mice in each group. Blood glucose value was determined using glucose dehydrogenase method. Then, 50 % glucose solution was administered (3 g/kg) to the animals for 30 and 60 min, respectively. The blood glucose level in oral glucose tolerance test (OGTT) was determined at 30-min intervals up to 120 min.

Insulin levels in the orbital venous plexus blood samples of mice were measured using Enzyme linked immunosorbent assay (ELISA), and HOMA-IR was calculated using Eq 1 where Fgb is Fasting blood glucose and fi is fasting insulin.

$$HOMA\text{-}IR = \frac{Fbg \times fi}{22.5} \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

The orbital venous plexus blood of mice in each group was collected and serum levels of lipid profiles were monitored with automatic biochemical analyzer. The mice in each group were decapitated and their ovarian tissues were taken. Total protein extraction from cells was done using RIPA buffer. The proteins were subjected to SDS-PAGE, followed by transfer to PVDF films which were blocked by incubation with 10 % skim milk. Thereafter, the films were incubated for 12 h at 4 ^{o}C with 1^{o} immunoglobulins, prior to TBST washing and incubation with HRP-conjugated secondary immunoglobulin at room temperature for 3 h. Then, the membranes were washed with TBST and the blots were subjected to ECL color development and imaging to determine protein expression levels of PI3K 85, AKT2, p-gsk-3 β Tyr216, p-GSK-3 βSer9 and GSK-3, withβ-actin as internal reference.

Statistical analysis

Measurement data with normal distribution of serum sex hormone levels of mice in each group are presented as mean ± SD. The snK-Q test was applied used for 2-group comparison, while multiple groups were compared with one-way ANOVA. All analyses were done with SPSS 23.0 software. Values of $p < 0.05$ indicated significant differences.

RESULTS

Serum sex hormone levels

The serum levels of 17-OHP, T and E2 were significantly higher in model mice than in control mice, but they were significantly reduced in berberine-treated mice, when compared to model mice. Serum 17-OHP level was comparable in all groups. These results are shown in Table 1.

OGTT blood glucose levels

At 60 min and 120 min, the OGTT blood glucose level was significantly higher in the model group than in blank control, but it was significantly lower in berberine dose groups than in model mice (Table 2).

Insulin levels and HOMA-IR

Compared with blank control group, HOMA-IR and fasting insulin level in model mice were significantly increased, but they were significantly lower in berberine dose groups than in model group ($p < 0.05$; Table 3).

Table 1: Levels of serum sex hormone in each group (mean ± SD, n = 16)

Group	17-OHP (ng/mL)	T (pg/mL)	E_2 (pmol/L)	*P* - value
Blank control	2.36±0.92	0.82±0.10	74.92±10.83	53.95±9.34
Model	3.64±1.42[a]	0.97±0.10[a]	99.37±23.68[a]	42.35±13.31
Low-dose berberine	2.55±0.79[b]	0.88±0.19[b]	81.33±8.79[b]	44.64±16.78
Medium-dose berberine	2.53±0.92[b]	0.87±0.10[b]	79.77±12.50[b]	48.21±14.27
High-dose berberine	2.50±0.72[b]	0.86±0.19[b]	78.24±12.21[b]	50.28±13.34

[a]$P < 0.05$, vs control, [b]$p < 0.05$, vs model. (*17-OHP= 17-hydroxyprogesterone; T = testosterone; E_2 = Estradiol)*

Table 2: OGTT blood glucose level in each group

Group	OGTT blood glucose level (mmol/L)			
	Empty stomach	30 min	60 min	120 min
Blank control	3.54±0.81	9.42±1.01	7.39±0.48	3.67±0.55
Model	3.72±0.48	11.73±0.96	9.53±1.06[a]	4.43±1.08[a]
Low-dose berberine	3.67±0.34	9.75±1.59	7.60±1.14[b]	4.08±0.67[b]
Medium-dose berberine	3.63±0.69	9.78±1.12	7.71±1.00[b]	4.28±0.57[b]
High-dose berberine	3.60±0.43	9.62±1.30	7.58±1.10[b]	4.20±0.85[b]

[a]$P < 0.05$, vs control; [b]$p < 0.05$, vs model

Table 3: Comparison of insulin levels and HOMA-IR in each group (mean ± SD, n = 16)

Group	Fasting insulin (pmol/L)	HOMA-IR
Blank control	1.06±0.41	0.26±0.17
Model	1.45±0.35[a]	0.32±0.16[a]
Low-dose berberine	1.21±0.26[b]	0.27±0.14[b]
Medium-dose berberine	1.17±0.27[b]	0.28±0.16[b]
High-dose berberine	1.20±0.29[b]	0.28±0.15[b]

[a]$P < 0.05$, vs control; [b]$p < 0.05$, vs model

Table 4: Levels of serum lipid metabolism-related indices in each group of mice (mean ± SD)

Group	TG (ng/mL)	TC (pg/mL)	LDL-C (pmol/L)	HDL-C (nmol/L)
Blank control	1.03±0.37	2.13±0.37	0.50±0.20	1.90±0.47
Model	1.81±0.49[a]	2.89±0.57[a]	0.68±0.24[a]	1.61±0.35
Low-dose berberine	1.24±0.33[b]	2.31±0.40[b]	0.56±0.23[b]	1.66±0.32
Medium-dose berberine	1.21±0.34[b]	2.24±0.28[b]	0.574±0.24[b]	1.72±0.35
High-dose berberine	1.09±0.36[b]	2.18±0.43[b]	0.52±0.25[b]	1.80±0.61

[a]$P < 0.05$, vs control; [b]$p < 0.05$, vs model

Levels of serum lipid metabolism-related indices

Serum concentrations of TG, TC and LDL-C in model mice were significantly raised, relative to control values. However, serum TG, TC and LDL-C in berberine dose groups were significantly lower than model group values (Table 4).

PI3K/Akt/GSK-3β signaling pathway-related protein expression levels

The protein concentrations of PI3K 85, Akt2 and P-GSK-3β Tyr216 in model group were significantly decreased, when compared to blank control group, but the protein expression levels of P-GSK-3β Ser9 were significantly raised, relative to control values. Protein expression levels of PI3K 85, Akt2 and P-GSK-3 β Tyr216 in berberine dose groups were significantly increased, relative to model mice, while the protein levels of P-GSK-3β Ser9 were significantly down-regulated, when compared to the model mice. The gSK-3β protein expression was comparable in all groups (Figure 1).

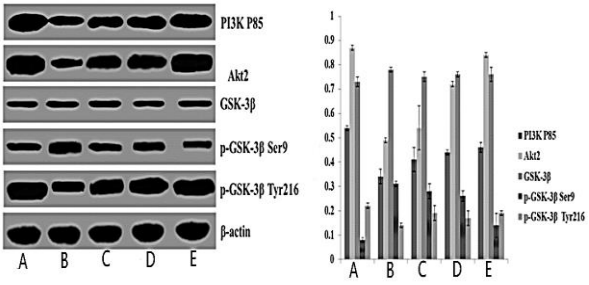

Figure 1: Comparison of PI3K/Akt/GSK-3β signal route-associated protein expression levels amongst the groups of mice. (A = Blank control; B = model; C, Low-dose berberine; D = medium-dose berberine. E = high-dose berberine)

DISCUSSION

At present, it is recognized that berberine accelerates glucose uptake and promotes the secretion of insulin. These factors are beneficial for relief of insulin resistance. Clinical studies have confirmed that berberine lowers blood lipids through improvement of the level of LDL-C in the liver [7]. Pharmacological studies have shown that berberine can be used as an insulin sensitizer, and for accelerating insulin release and improving glucose metabolism [8].

Abnormal rise in insulin level leads to increases in liver sex hormone binding globulin, free and testosterone levels, and increased secretion of pituitary luteinizing hormone. At the same time, the release of follicle hormone presents negative feedback adjustment to enhance and maintain the luteinizing hormone effect. This indirectly causes ovarian androgen release and corresponding sex hormone synthesis, eventually leading to disorders in egg production [9]. Therefore, reduction of excessive serum insulin and androgen levels in PCOS patients are key to treatment of the disease.

This research has demonstrated that serum levels of 17-OHP, T and E2 in berberine dose groups were significantly reduced, relative to model mice values. This suggests that the model group mice had hyperandrogenemia, but the endocrine function of mice was improved significantly after berberine intervention. A study has reported that berberine improved the glucose consumption capacity of hepatocyte HepG2 in an insulin-independent way, and that the intensity of the effect was equivalent to that of metformin [10]. In addition, berberine did not affect insulin release. The OGTT blood glucose levels were significantly reduced in berberine dose groups, when compared with values in model mice. These data suggest that berberine relieved the abnormal glucose metabolism of PCOS mice and accelerated glucose metabolism. Thus, it may be used for the treatment of abnormal glucose metabolism in PCOS patients in the clinic.

Fasting insulin level and HOMA-IR were significantly lower in berberine-exposed mice than in model mice, suggesting that berberine significantly reduced fasting insulin content and mitigated insulin insensitivity. Usually, HOMA-IR is used to analyze insulin resistance, and some patients have abnormal blood lipid and lipoprotein levels, as indicated by raised lipid profiles, indicating that PCOS is a metabolic

disease. Therefore, these indices were selected in this study to analyze the lipid metabolism of mice. There were significantly lower levels of lipid parameters in berberine dose groups than in model mice. These results indicate that berberine has the potential to regulate lipid metabolism of PCOS patients.

Berberine promotes the level of peroxisome proliferating-activated receptor subunit, and regulates blood lipid at multiple levels. An increase in the level of this subunit may be one of the mechanisms associated with regulation of plasma lipids by berberine [11]. Xiao [12] reported that berberine accelerated cholesterol reverse-transport and fatty acid oxidation, and reduced serum TG and TC contents. Studies have shown the relevance of PI3K in insulin metabolism and mitosis, with P85 is one of its regulatory subunits [13,14]. One of the subtypes of Akt which is located downstream of PI3K pathway, is Akt2. It has been found that Akt2 accelerated cell growth and maintained normal cell growth [15], especially under the physiological effects of insulin and other types of growth factors [13].

Several tissues express GSK-3 β. A study reported that insulin blocked GSK-3β activity, mediated Akt2 phosphorylation of GSK-3, and induced GS dephosphorylation, thereby improving its activity and ultimately accelerating glycogen synthesis and reducing blood glucose levels [14,16]. The activity of GSK-3β is determined by phosphorylation at Ser9 and Tyr216: it is blocked by phosphorylation at Tyr216, and activated by phosphorylation at Ser9 [15]. The results obtained in this study indicated significantly up-regulated proteins of PI3K 85, Akt2 and P-GSK-3 β Tyr216 in berberine dose mice, relative to model mice, but P-GSK-3β Ser9 protein levels in berberine dose mice were significantly reduced, relative to model value. These results indicate that the PI3K/Akt/GSK-3β insulin signaling pathway was blocked in the ovary of PCOS mice, resulting in local ovarian insulin resistance. However, after berberine intervention, the local ovarian insulin resistance of PCOS mice was significantly reduced.

CONCLUSION

Berberine improved endocrine function and local insulin resistance of the ovary in PCOS mice, and also improved insulin sensitivity, possibly through benign regulation of vital proteins of PI3K/Akt/GSK-3β insulin signal route.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was performed by the authors named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Yaxin Liu designed the study, supervised the data collection, and analyzed the data. Dan Gao interpreted the data and prepared the manuscript for publication. Dan Gao supervised the data collection, analyzed the data and reviewed the draft of the manuscript.

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