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Effects of Guizhi Fuling Pill combined with metformin on insulin resistance−related inflammatory response and oxidative stress response in patients with PCOS

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ABSTRACT

Objective: To study the effects of Guizhi Fuling Pill combined with metformin on insulin resistance-related inflammatory response and oxidative stress response in patients with polycystic ovary syndrome (PCOS). **Methods:** PCOS patients who received therapy in Panzhihua Maternal and Child Care Service Centre between June 2013 and February 2013 were selected and randomly divided into two groups, combined therapy group received Guizhi Fuling Pill combined with metformin therapy for two months, and metformin group received metformin therapy for continuous two months. After serum pickup, the follicular fluid was collected to determine the expression of insulin signaling pathway molecules as well as the protein levels of inflammatory response indexes and oxidative stress response indexes. **Results:** Serum IRS-1, IRS-2, PI3K, AKT and GLUT4 mRNA expression of combined therapy group were greatly higher than those of metformin group whereas GSK-3β mRNA expression was significantly lower than that of metformin group; serum NF-kB, TNF-α, IL-6, IL-18, MCP-1, ROS, MDA and 8-OHdG protein levels of combined therapy group were greatly below those of metformin group and negatively correlated with IRS-1 and IRS-2 mRNA expression whereas TAC and SOD levels were higher than those of metformin group and positively correlated with IRS-1 and IRS-2 mRNA expression. **Conclusion:** Guizhi Fuling Pill combined with metformin can reduce the inflammatory response and oxidative stress response to improve the insulin resistance and increase the insulin sensitivity in patients with PCOS.

1. Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disorder in adolescent girls and women of childbearing age, which is mainly characterized by oligomenorrhea, hypertrichosis, acne and obesity. Insulin resistance is a prominent pathological feature of PCOS. There is a significant hyperinsulinemia, which can lead to abnormal lipid metabolism and increase the risk of type 2 diabetes mellitus, cardiovascular and cerebrovascular diseases, etc. At present, the mechanism of insulin resistance in patients with PCOS is still not completely clear, but the relationship of chronic inflammation and oxidative stress response with insulin resistance has been confirmed by several studies[1−3]. Metformin is a drug with insulin sensitization effect, which is widely used in the treatment of PCOS, can effectively increase insulin sensitivity[4,5], but has no significant regulatory effect on the insulin biological signal transduction as well as the inflammation and oxidative stress reaction that cause insulin resistance. Guizhi Fuling Pill is a Chinese patent drug for PCOS, and it has the functions of activating blood circulation to remove stasis and regulating endocrine balance. In the following studies, we specifically analyzed the effects of Guizhi Fuling Pill combined with metformin on insulin resistance-associated inflammatory response and oxidative stress in PCOS patients.

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2. General information and research methods

2.1 General case information

PCOS patients who received therapy in Panzhihua Maternal and Child Care Service Centre between June 2013 and February 2013 were selected as the research subjects, all the patients conformed to the diagnostic criteria for PCOS, and the patients combined with adrenal gland, thyroid gland, pituitary gland and other endocrine gland dysfunction were ruled out. A total of 70 patients were enrolled and divided into two groups by random number table, 35 cases in each group. The combined therapy group was 32-41 years old, the infertility course was 2-7 years, and the BMI was (25.2±3.6) kg/m^2; metformin group were 33-40 years old, the infertility course was 2-8 years, and the BMI was (25.8±3.3) kg/m^2. There was no statistically significant difference in general information between the two groups (P>0.05).

2.2 Therapy

Metformin group received metformin hydrochloride tablets, and the method was as follows: metformin hydrochloride tablets 0.5 g, taken orally, 3 times/d. Combined therapy group received Guizhi Fuling Pill combined with metformin hydrochloride tablets therapy, which was as follows: Guizhi Fuling Pill 6 g, taken orally, 2 times/d; metformin hydrochloride tablets 0.5 g, taken orally, 3 times/d. Both groups were treated for two consecutive months.

2.3 mRNA expression detection

After 2 months of treatment, peripheral blood was collected, the kits were used to extract RNA and synthesize it into cDNA by reverse transcription, the specific primers for IRS-1, IRS-2, PI3K, AKT, GLUT4 and GSK-3β gene were designed, the primers and fluorescence quantitative PCR kit were used for reaction, and the corresponding gene mRNA expression was determined.

2.4 Protein level detection

After 2 months of treatment, peripheral blood was collected, enzyme-linked immunosorbent assay kit was used to determine the contents of NF-kB, TNF-α, IL-6, IL-18 and MCP-1, and radioimmunoprecipitation kit was used to detect the contents of ROS, MDA, 8-OHdG, TAC and SOD.

2.5 Statistical methods

SPSS 20.0 software was used for the t test of the differences in data between two groups as well as the Pearson test of the correlation between two data, and P<0.05 meant statistical significance in differences in test results.

3. Results

3.1 Serum insulin signaling pathway molecule expression

After 2 months of treatment, analysis of serum insulin signaling pathway molecules IRS-1, IRS-2, PI3K, AKT, GLUT4 and GSK-3β expression between two groups of patients was as follows: serum IRS-1, IRS-2, PI3K, AKT and GLUT4 mRNA expression of combined therapy group were greatly higher than those of metformin group whereas GSK-3β mRNA expression was significantly lower than that of metformin group.

3.2 Serum inflammatory response index levels and their correlation with IRS-1 and IRS-2

After 2 months of treatment, analysis of serum inflammatory response indexes NF-kB (ng/mL), TNF-α (ng/mL), IL-6 (ng/mL), IL-18 (pg/mL) and MCP-1 (pg/mL) levels between two groups of patients was as follows: serum NF-kB, TNF-α, IL-6, IL-18 and MCP-1 protein levels of combined therapy group were greatly below those of metformin group. Pearson test showed that serum NF-kB, TNF-α, IL-6, IL-18 and MCP-1 protein levels of combined therapy group were negatively correlated with IRS-1 and IRS-2 mRNA expression.

Table 1.

Comparison of serum insulin signaling pathway molecules.

Groups	n	IRS-1	IRS-2	PI3K	AKT	GLUT4	GSK-3β
Combined therapy group	35	2.62±0.39	2.33±0.37	1.93±0.26	2.92±0.46	2.42±0.39	0.35±0.08
Metformin group	35	1.03±0.17	1.01±0.15	1.04±0.19	0.97±0.14	0.99±0.11	1.05±0.17
t		17.498	12.586	9.395	22.109	15.689	19.39482
P		<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

Table 2.

Comparison of serum inflammatory response indexes.

Groups	n	NF-kB	TNF-α	IL-6	IL-18	MCP-1
Combined therapy group	35	4.95±0.78	2.03±0.35	1.87±0.25	231.92±33.58	189.31±22.48
Metformin group	35	10.27±1.42	5.61±0.78	4.21±0.59	573.94±64.95	428.97±72.39
t		13.294	15.219	12.697	14.622	11.958
P		<0.05	<0.05	<0.05	<0.05	<0.05

Table 3.

Comparison of serum oxidative stress response indexes.

Groups	*n*	ROS	MDA	8-OHdG	TAC	SOD
Combined therapy group	35	1.02±0.16	2.03±0.36	10.25±1.35	70.32±9.35	98.41±10.25
Metformin group	35	1.88±0.27	5.69±0.78	28.69±3.86	31.25±4.58	39.61±4.96
t		8.498	14.282	17.039	11.938	13.458
P		<0.05	<0.05	<0.05	<0.05	<0.05

3.3 Serum oxidative stress response index levels and their correlation with IRS-1 and IRS-2

After 2 months of treatment, analysis of serum oxidative stress response indexes ROS (U/mL), MDA (nmol/mL), 8-OHdG (ng/mL), TAC (nmol/mL) and SOD (U/mL) between two groups of patients was as follows: serum ROS, MDA and 8-OHdG levels of combined therapy group were greatly below those of metformin group whereas TAC and SOD levels were higher than those of metformin group. Pearson test showed that serum ROS, MDA and 8-OHdG levels of combined therapy group were negatively correlated with IRS-1 and IRS-2 mRNA expression whereas TAC and SOD levels were positively correlated with IRS-1 and IRS-2 mRNA expression.

4. Discussion

Insulin resistance and hyperinsulinemia are the important features of PCOS patients, insulin sensitizer metformin is a common drug for PCOS, and it can effectively relieve insulin resistance and increase insulin sensitivity[6,7]. Guizhi Fuling Pill is a Chinese patent medicine that has been used in the treatment of PCOS in recent years, which contains Chinese herbs such as cassia twig, Poria cocos, cortex moutan and peach seed, and can promote blood circulation to remove blood stasis and balance Yin and Yang. IRS-1 and IRS-2 are the phosphorylated proteins involved in insulin biosignal transduction. The decrease of IRS-1 and IRS-2 expression in vivo is the important cause of insulin resistance in patients with PCOS[8]. Under physiological conditions, the combination between insulin and receptors on cell membrane can cause the tyrosine phosphorylation of receptors themselves, and then the IRS-1 and IRS-2 are combined with the intracellular fragments of insulin receptor and phosphorylated, activating downstream cascade signal transduction process[9,10]. PI3K/AKT pathway is an important downstream signaling pathway of IRS-1 and IRS-2, and its activation can on the one hand, increase GLUT-4 expression and promote peripheral tissue to take in glucose, and on the other hand, cause the GSK-3β phosphorylation inactivation and promote the synthesis of glycogen[11,12]. The changes in above insulin signaling pathway molecule expression in serum were analyzed in the study to reflect the degree of insulin resistance, and the results showed that serum IRS-1, IRS-2, PI3K, AKT and GLUT4 mRNA expression of combined therapy group were greatly higher than those of metformin group while GSK-3β mRNA expression was significantly lower than that of metformin group. It means that Guizhi Fuling Pill combined with metformin therapy is more effective than metformin monotherapy to activate the insulin signaling pathway mediated by IRS-1 and IRS-2, then increase the insulin sensitivity and reduce the insulin resistance in patients with PCOS.

During the occurrence of insulin resistance, the continuous activation of chronic inflammatory response and the continuous secretion of inflammatory mediators are the important pathological links that affect insulin sensitivity. The activation of inflammatory response and the secretion of inflammatory mediators can directly affect the phosphorylation activation process of IRS-1 and IRS-2 to impede the insulin biological signal transduction and cause insulin resistance. NF-kB is a key transcription factor regulating inflammatory response, and the activated NF-kB can dissociate with inhibitor IkB and enter the nucleus to initiate the transcription of various inflammatory factors[13,14]. TNF-α is an inflammatory factor secreted by mononuclear macrophages, IL-6 is a multifunctional cytokine secreted by monocytes and lymphocytes, and both can inhibit the phosphorylation of IRS-1 and IRS-2; IL-18 is a cytokine with pro-inflammatory activity[15,16], which can activate multiple inflammatory cells; MCP-1 is a cytokine with chemotaxis effect and can enable multiple inflammatory factors to infiltrate in the local lesion, and the two can influence the transduction of insulin signaling pathway by amplifying inflammatory response[17,18]. Analysis of the differences in inflammatory response indicators in the serum of the two groups showed that serum NF-kB, TNF-α, IL-6, IL-18 and MCP-1 protein levels of combined therapy group were greatly lower than those of metformin group. This indicates that the Guizhi Fuling Pill combined with metformin therapy can be more effective than metformin therapy alone to inhibit the inflammatory response in PCOS patients. The correlation of inflammatory response with IRS-1 and IRS-2 expression was further analyzed, and the results showed that serum NF-kB, TNF-α, IL-6, IL-18 and MCP-1 protein levels of combined therapy group were negatively correlated with IRS-1 and IRS-2 mRNA expression. This means that the inhibition of inflammation in patients with PCOS is associated with the increase of IRS-1 and IRS-22 expression, and Guizhi Fuling Pill combined with metformin therapy can inhibit inflammatory reaction to increase the IRS-1 and IRS-22 expression as well as insulin sensitivity.

The insulin resistance in patients with PCOS is not only associated with inflammatory reaction activation, but also closely related to oxidative stress response. Increased formation of ROS is an important characteristic of oxidative stress response activation, and hyperinsulinemia has stimulating effects on ROS generation in the local tissue and cells, which can promote the oxidative stress reaction activation[19,20]. MDA and 8-OHdG are the reaction products of lipid and nucleic acid in local tissues and cells with ROS respectively, which can reflect the production of ROS and the degree of oxidative stress response. In the process of constant ROS production, the antioxidase SOD in vivo will be constantly consumed, which leads to the reduction of TAC[21,22]. Analysis of the differences in

serum oxidative stress indicators between two groups of patients showed that serum ROS, MDA and 8-OHdG levels of combined therapy group were greatly below those of metformin group while TAC and SOD levels were higher than those of metformin group. This indicates that Guizhi Fuling Pill combined with metformin therapy can more effectively inhibit the oxidative stress response in PCOS patients than metformin monotherapy. Further analysis of the correlation of oxidative stress reaction with IRS-1 and IRS-22 expression showed that serum ROS, MDA and 8-OHdG levels of combined therapy group were negatively correlated with IRS-1 and IRS-2 mRNA expression while TAC and SOD levels were positively correlated with IRS-1 and IRS-2 mRNA expression. This means that the inhibition of oxidative stress responses in patients with PCOS is associated with the increase of IRS-1 and IRS-22 expression, and Guizhi Fuling Pill combined with metformin therapy can inhibit oxidative stress reaction to increase the IRS-1 and IRS-22 expression as well as insulin sensitivity.

The above analysis of insulin signal transduction as well as related inflammation and oxidative stress shows that Guizhi Fuling Pill combined with metformin is more effective than metformin alone to increase the insulin sensitivity of patients with PCOS, and reducing inflammation and oxidative stress reaction is the possible mechanism for combined therapy to improve insulin resistance and increase insulin sensitivity.

References

[1] Kalyan S, Patel MS, Kingwell E, Cote HCF, Liu D, Prior JC. Competing factors link to bone health in polycystic ovary syndrome: chronic low-grade inflammation takes a toll. *Sci Rep* 2017; **7**(1): 3432.

[2] Milutinovic DV, Nikolic M, Velickovic N, Djordjevic A, Bursac B, Nestorov J, et al. Enhanced inflammation without impairment of insulin signaling in the visceral adipose tissue of 5 -dihydrotestosterone-induced animal model of polycystic ovary syndrome. *Exp Clin Endocrinol Diabetes* 2017; **125**(8): 522-529.

[3] Saglam E, Canakcı CF, Sebin SO, Saruhan N, Ingec M, Canakcı H, et al. Evaluation of oxidative status in patients with chronic periodontitis and polycystic ovary syndrome: a cross-sectional study. *J Periodontol* 2017; **28**: 1-16.

[4] Practice Committee of the American Society for Reproductive Medicine. Electronic address: ASRM@asrm.org; Practice Committee of the American Society for Reproductive Medicine. Role of metformin for ovulation induction in infertile patients with polycystic ovary syndrome (PCOS): a guideline. *Fertil Steril* 2017; **108**(3): 426-441.

[5] Kumar DRN, Seshadri KG, Pandurangi M. Effect of Metformin-sustained release therapy on low-density lipoprotein size and adiponectin in the south indian women with polycystic ovary syndrome. *Indian J Endocrinol Metab* 2017; **21**(5): 679-683.

[6] Al-Ruthia YS, Al-Mandeel H, AlSanawi H, Balkhi B, Mansy W, AlGasem R, et al. The effect of metformin use on pregnancy rates among polycystic ovary syndrome patients undergoing in vitro fertilization: A retrospective-cohort study. *Saudi Pharm J* 2017; **25**(6): 906-910.

[7] Esmaeilzadeh S, Gholinezhad-Chari M, Ghadimi R. The effect of metformin treatment on the serum levels of homocysteine, folic acid, and vitamin b12 in patients with polycystic ovary syndrome. *J Hum Reprod Sci* 2017; **10**(2): 95-101.

[8] Hao M, Yuan F, Jin C, Zhou Z, Cao Q, Xu L, et al. Overexpression of lnk in the ovaries is involved in insulin resistance in women with polycystic ovary syndrome. *Endocrinology* 2016; **157**(10): 3709-3718.

[9] Shi X, Xie X, Jia Y, Li S. Associations of insulin receptor and insulin receptor substrates genetic polymorphisms with polycystic ovary syndrome: A systematic review and meta-analysis. *J Obstet Gynaecol Res* 2016; **42**(7): 844-854.

[10] Kaur S, Anjali G, Bhardwaj P, Taneja J, Singh R. Data in support of FSH induction of IRS-2 in human granulosa cells: Mapping the transcription factor binding sites in human IRS-2 promoter. *Data Brief* 2015; **13**(6): 162-167.

[11] Yaluri N, Modi S, Kokkola T. Simvastatin induces insulin resistance in L6 skeletal muscle myotubes by suppressing insulinsignaling, GLUT4 expression and GSK-3 β phosphorylation. *Biochem Biophys Res Commun* 2016; **480**(2): 194-200.

[12] Li W, Liang X, Zeng Z, Yu K, Zhan S, Su Q, et al. Simvastatin inhibits glucose uptake activity and GLUT4 translocation through suppression of the IR/IRS-1/Akt signaling in C2C12 myotubes. *Biomed Pharmacother* 2016; **83**: 194-200.

[13] Zuo T, Zhu M, Xu W, Wang Z, Song H. Iridoids with genipin stem nucleus inhibit lipopolysaccharide-induced inflammation and oxidative stress by blocking the nf- κ b pathway in polycystic ovary syndrome. *Cell Physiol Biochem* 2017; **43**(5): 1855-1865.

[14] Koc O, Ozdemirici S, Acet M, Soyturk U, Aydin S. Nuclear factor-κ B expression in the endometrium of normal and overweight women with polycystic ovary syndrome. *J Obstet Gynaecol* 2017; **37**(7): 924-930.

[15] Daan NM, Koster MP, de Wilde MA, Dalmeijer GW, Evelein AM, Fauser BC, et al. Biomarker profiles in women with pcos and PCOS offspring; a pilot study. *PLoS One* 2016; **11**(11): e0165033.

[16] Wu H, Yu K, Yang Z. Associations between TNF- and interleukin gene polymorphisms with polycystic ovary syndrome risk: a systematic review and meta-analysis. *J Assist Reprod Genet* 2015; **32**(4): 625-634.

[17] Yousuf SD, Rashid F, Mattoo T, Shekhar C, Mudassar S, Zargar MA, et al. Does the oral contraceptive pill increase plasma intercellular adhesion molecule-1, monocyte chemoattractant protein-1, and tumor necrosis factor-α levels in women with polycystic ovary syndrome: a pilot study. *J Pediatr Adolesc Gynecol* 2017; **30**(1): 58-62.

[18] Long X, Li R, Yang Y, Qiao J. Overexpression of IL-18 in the proliferative phase endometrium of patients with polycystic ovary syndrome. *Reprod Sci* 2017; **24**(2): 252-257.

[19] Victor VM, Rovira-Llopis S, Banuls C, Diaz-Morales N, Martinez de Marañon A, Rios-Navarro C, et al. Insulin resistance in PCOS patients enhances oxidative stress and leukocyte adhesion: role of myeloperoxidase. *PLoS One* 2016; **11**(3): e0151960.

[20] Hyderali BN, Mala K. Oxidative stress and cardiovascular complications in polycystic ovarian syndrome. *Eur J Obstet Gynecol Reprod Biol* 2015; **191**: 15-22.

[21] Pekel A, Gonenc A, Turhan NO, Kafali H. Changes of sFas and sFasL, oxidative stress markers in serum and follicular fluid of patients undergoing IVF. *J Assist Reprod Genet* 2015; **32**(2): 233-241.

[22] Sumithra NU, Lakshmi RL, Leela Menon N, Subhakumari KN, Sheejamol VS. Evaluation of oxidative stress and hscrp in polycystic ovarian syndrome in a tertiary care hospital. *Indian J Clin Biochem* 2015; **30**(2): 161-166.