Effect of Alpha Lipoic Acid on Polycystic Ovary Syndrome with Insulin Resistance

Budi Santoso[1], Rusnaidi[2], Widjiati[3]

[1]Professor, [2]Student in Department of Obstetrics and Gynecology, Faculty of Medicine Universitas Airlangga – Dr. Soetomo Teaching Hospital, Surabaya, Indonesia, [3]Professor in Department of Veterinary Reproduction, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia

Abstract

Background: Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in reproductive age women where insulin resistance plays an important role. Insulin resistance makes the first line treatment, clomiphene citrate (CC) treatment, become ineffective. Alpha Lipoic Acid (ALA) is believed to be an alternative treatment for CC-resistant PCOS. **Objectives:** The aim of the study is to understand the effect of ALA in Insuline receptor substrate 1 (IRS-1) expression, Glucosa transporter 4 (GLUT-4) expression, and folliculogenesis in insulin resistant PCOS rat model. **Methods:** This was an experimental study with randomized posttest only control group design. 30 females rat injected with testosterone propionate (TP) 1mg/100gram bodyweight for 28 days then divided into 3 groups. Negative control group receive no other treatment, positive control group receive a placebo for 14 days, and treatment group receive ALA for 14 days. IRS-1 and GLUT-4 expression is evaluated with immunohistochemistry, while folliculogenesis is evaluated by counting the number of follicle in each stage. **Results:** Mean IRS-1 expression in muscle in treatment group is significantly higher than other groups (4.28±1.05; 3.02±1.03; 1.86±0.83, $p < 0.01$ respectively). Mean GLUT-4 expression in treatment group is significantly higher than other groups (4.28±0.91; 3.20±1.14; 1.40±0.55, $p < 0.01$ respectively). Mean number of follicle in each stage in treatment group are significantly reduced than other groups (all $p < 0.05$). **Conclusion:** ALA increase the expression of IRS-1 and GLUT-4, and also reduce the number of follicle in each stage of folliculogenesis**.**

Keywords: *PCOS, Alpha Lipoic Acid, IRS-1, GLUT-4, Folliculogenesis*

Introduction

Polycystic Ovary Syndrom (PCOS) is the most common endocrine disorder in reproductive age woman with prevalence around 5-21%[1]. Female with PCOS have high incidence of insulin resistance with prevalence of 50-75% and decrease insulin sensitivity about 35-40% than normal[2]. Insulin resistance plays an important pathogenic role in hyperandrogen both for obese and lean female PCOS, accompanied by hyperinsulinemia and increases the risk of type 2 diabetes mellitus (DM)[3]. Infertility treatment with clomiphene citrate (CC) is the first-line therapy for ovulation induction, but there is CC resistance of 15-40% where insulin, hyperandrogen and obesity resitance are the major factors[4].

Basal insulin therapy could increase the glicemic control[5]. However, consuming supplement, such as vitamin C and vitamin E, could not increasing insulin in the blood[6]. Previous study found that consuming *Moringa oleifera* decrease level of blood insulin and androgen, so that folliculogenesis could increase[7]. Metformin is one of the insulin sensitizer that often used for PCOS therapy with insulin resistance. Although metformin may decrease insulin resistance, it has undesirable side effects such as gastrointestinal disorders, thus a long-

Corresponding Author:
Budi Santoso,
Department of Obstetrics and Gynecology, Faculty of Medicine Universitas Airlangga – Dr. Soetomo Teaching Hospital,
Email: budisantosounair@gmail.com

term use may decrease patient compliance[8]. Because of its side effect and it is important to decrease insulin resistance in PCOS, it is necessary to use alternative treatment that can reduce insulin resistance and increase the incidence of ovulation and pregnancy rates by using alpha lipoic acid (ALA). ALA is a powerful antioxidant, detoxifying agent and diabetes mellitus drug. It also has been involved as a modulator of inflammatory signal pathways[9]. ALA can improve glucose control in DM type 2, with its mechanism on decreasing oxidative stress and insulin resistance, and increasing peripheral insulin sensitivity[10,11].

Thus, this study aims to determine whether ALA is able to improve insulin resistance and follicologenesis in PCOS with insulin resistance with Insuline receptor substrate 1 (IRS-1), Glucosa transporter 4 (GLUT-4), and follicular as parameters.

Methods

This was an experimental study with randomized posttest only control group design. This study was done in Faculty of Veterinary, Universitas Airlangga in February-may 2017. The sample in this study was *Rattus norvegicus* strain wistar female rat around 4-5 months old. The inclusion criterias for animal model were 4 - 5 months old, 150 - 260 gram weight, female gender, healthy (characterized by active movement, glowing eyes), and received injection of 1 mg / 100 gr BB testosterone propionate subcutaneously during 28 days. While the animal models included in the exclusion criteria were diseased, defective or congenital and aggressive (seen in 2x24 hours of observation with parameters often attacking other group members). While the dropout criteria are wounded model, die before, during and after administration of ALA, prior to the examination.

Samples were taken from population by randomization. The models were divided into 3 groups, one group of PCOS model with insulin resistance given ALA for 14 days (treatment group), one group of PCOS model with insulin resistance which only given placebo for 14 days (positive control group), and one group of PCOS model with insulin resistance without any treatment (negative control group). The sample size was calculated using Federer's formula and with 10% addition to prevent drop out, thereby 10 models were obtained for each group. This study was ethically approved by Ethics Committee of Faculty of Veterinary Medicine, Universitas Airlangga before conducting the study. All experiments were performed in accordance with relevant regulations.

This study used level of significance 0.05, means if statistical test obtained p-value $\leq$ 0.05 it means significance. All data homogeneity were tested with Levene test to assess whether there was any difference of variance between the three groups. Anova test was used to analyze the difference in IRS-1 expression, GLUT-4 expression, and number of follicle in every stages of folliculogenesis. Multiple linear regression analysis was used to analyze the effect of ALA, IRS-1, and GLUT-4 on folliculogenesis. All data analyses were performed using SPSS version 17.0 (SPSS, Inc., Chicago IL).

Results

Sample Characteristic

In this study, all samples weight were analyzed using Levene test and obtained homogenous data variation. Using ANOVA test, the result showed no significance differences between treatment, positive control and negative control groups weight (207.50±42.51; 204.50±19.07; 208±35.84, p = 0.97 respectively).

IRS-1 Expression

The homogeneity test of IRS-1 expression in muscle using Levene test obtained a homogeneous data variance. Then, using the Anova test, a significant differences in IRS-1 expression were found between the treatment group, positive control, and negative control (4.28±1.05; 3.02±1.03; 1.86±0.83, p <0.01 respectively) (Table 1). The homogeneity test of IRS-1 expression in ovaries using Levene test obtained homogeneous data variance. Using the Anova test, significant differences in IRS-1 expression were found between the treatment group, positive control, and negative control (7.78±1.10; 3.06±1.56; 2.46±0.89, p <0.01 respectively) (Table 1).

GLUT-4 Expression in Muscle

The homogeinity test of GLUT-4 variable using Levene test obtained homogeneous data variance. The ANOVA test showed GLUT-4 expression was significantly differences between the treatment group,

positive control, and negative control (4.28±0.91; 3.20±1.14; 1.40±0.55, p <0.01 respectively) (Table 1).

Folliculogenesis

The homogeneity test of primary follicles number variable using Levene test obtained an inhomogeneous variance data. The ANOVA test showed a significance differences in primary follicle number between the treatment group, positive control, and negative control (58±13.11; 66±19.81; 92±44.9, p = 0.04 respectively) (Table 2). The homogeneity test result of secondary follicles number variable using Levene test was homogeneous. The ANOVA test showed tertiary follicle number was significantly difference between the treatment group, positive control, and negative control (18±4.62; 27±10.82; 44±16.6, p <0.01 respectively) (Table 2).

The homogeneity test result of tertiary follicles number variable using Levene test was homogeneous. The ANOVA test showed a significance difference in secondary follicle number between the treatment group, positive control, and negative control (2±1.56; 4±1.89; 7±4.37, p <0.01 respectively) (Table 2). The homogeneity test of de Graaf follicles number using Levene test obtained an inhomogeneous variance data. The Kruskal walls test showed a significance difference in de Graaf follicles number between the treatment group, positive control, and negative control (5±3.37; 2±0.82; 0.4±0.52, p <0.01 respectively) (Table 2).

The homogeneity test result of corpus luteum number using Levene test was homogeneous. The ANOVA test showed a significance difference in corpus luteum number between the treatment group, positive control, and negative control (9±3.37; 6.8±3.58; 2±2.36, p <0.01 respectively) (Table 2).

ALA, IRS-1 and GLUT-4 effects on folliculogenesis

Using multiple linear regression analysis, we found that ALA, IRS-1 in muscle, IRS-1 in ovaries and GLUT-4 had an effect on the number of each phase of folliculogenesis, and especially on the number of De Graff follicles (Table 3).

Table 1. The difference in expression of IRS-1 in muscle, IRS-1 in ovarium, and GLUT-4 in muscle between groups

	Treatment group	Positive control group	Negative control group	p
IRS-1 in muscle	4.28 ± 1.05	3.02 ± 1.03	1.86 ± 0.83	<0.01
IRS-1 in ovarium	7.78 ± 1.10	3.06 ± 1.56	2.46 ± 0.89	<0.01
GLUT-4 in muscle	4.28 ± 0.91	3.20 ± 1.14	1.40 ± 0.05	<0.01

IRS-1= *Insulin Receptor Substrate-1*; GLUT-4= *Glucose Transporter Type-4*. Anova test was used. A p value of <0.05 was considered significant

Table 2. The differences in number of follicle between groups

	Treatment group	Positive control group	Negative control group	p
Number of Primary Follicle	58 ± 13.11	66 ± 19.81	92 ± 44.90	0.04
Number of Secondary Follicle	18 ± 4.62	27 ± 10.82	44 ± 16.6	<0.01
Number of Tertiary Follicle	2 ± 1.56	4 ± 1.89	7 ± 4.37	<0.01
Number of de Graaf Follicle	5 ±3.37	2 ± 0.82	0.40 ± 0.52	<0.01
Number of Corpus Luteum	9 ± 3.37	6.8 ± 3.58	2 ± 2.36	<0.01

Anova test was used. A p value of <0.05 was considered significant

Table 3. The Effect of Alpha Lipoic Acid, IRS-1 in muscle, IRS-1 in ovarium, and GLUT-4 on Folliculogenesis

Dependent Variable	F	Adjusted R Squared	P
Number of Primary Follicle	2.69	0.226	0.05
Number of Secondary Follicle	6.40	0.482	<0.01
Number of Tertiary Follicle	3.62	0.311	0.01
Number of de Graff Follicle	6.44	0.484	<0.01
Number of Corpus Luteum	5.18	0.419	<0.01

Multiple linear regression analysis was used. A p value of <0.05 was considered significant

Discussion

This study result obtained the IRS-1 expression in muscle of the treatment group was significantly different than the positive control and negative control. Increasing IRS-1 expression was caused by ALA as a potent antioxidant, which capable of binding various species of reactive oxygen species (ROS). Chronic oxidative stress induces a number of insulin signaling pathways, especially via the *nuclear factor* kappa-light-chain-enhancer of activated B cells (NFκB) and c-Jun N-terminal kinases/ stress-activated kinases (JNK/SAPK) pathways, which could change the phosphorylation of serine into tyrosine phosphorylation (shay). Furthermore, ALA can bind directly and activate the tyrosine kinase domain in insulin receptor (IR) subunit-β resulting the IR becomes active. ALA effects on IR/IRS-1 will increase the combination of IRS-1 and *Phosphoinositide* 3-kinases (PI3K), also increase PI3K activity in the membrane environment. Then activating Akt to regulate the GLUT-4 displacement between storage vesicles and plasma membranes through mechanisms involving AS160 phosphorylation. ALA induces IRS-1 phosphorylation,

IRS-1/PI3K signal activation and stimulates GLUT-4 translocation to cell membranes through inactivation of Akt 160 (AS160) substrate[9]. Hence, ALA will increase insulin signal activation through IRS-1 enhancement and increase glucose uptake by increasing GLUT-4 to reduce insulin resistance.

In this study, there were significant differences of GLUT-4 expression between treatment group and control group. This suggests that ALA administration significantly increases GLUT-4 muscle expression in PCOS animal models with insulin resistance. The main function of GLUT-4 is to facilitate the glucose intake into muscle cells and adipocyte and also maintain control of blood glucose level. Insulin resistance may result from interruption of insulin signal transduction causing GLUT-4 translocation to decrease.The translocation of insulin-mediated GLUT-4 into the plasma membrane includes the PI3K complex[12].

This study obtained a significant differences in each phase of folliculogenesis between the treatment and the control group. The data showed decrease dominancy of primary, secondary and tertiary follicles, and significant increase in the number of de Graaf follicles and corpus luteum treatment group. ALA can decrease insulin resistance and lowered androgen levels thus improving the selection stage of follicugenesis by increasing the number of De Graaf follicles. However, it did not cause ovulation, because pre-ovulation estrogen levels were inadequate to trigger Lutenizing hormone (LH) surges, resulting in no ovulation. Ovulation will likely occurs if ALA dosing was raised and the administration was extended. The estrous cycle in rats is divided into 4 different phases compared to humans: Proestrus, estrus, metestrus, and diestrus. The proestrus and estrus phases resemble the follicular phase, while the metestrus and diestrus phases resemble the luteal phase in humans. The markers of ovarian development in mice resemble humans, only the timing is shorter. A number of primordial follicles will develop into primary follicles. Factors that affect the development at this stage is still not fully understood. A new Follicle-stimulating hormone (FSH) functional receptors are formed at the secondary follicle stage. The development of the preantral follicles (primary and secondary) is affected by FSH but does not depend entirely. The pre-antral follicle then enters the cyclic recruitment into the antral follicle (tertiary) and eventually becomes the preovulatory follicle (de Graaf follicle).

The development of antral follicle is highly dependent on FSH. Only follicle that has sufficient number of FSH receptors will capable of developing into de Graaf follicle and ovulate. Corpus luteum is a mass of ovaries formed by ovulatory ovulation. Rats formed more than one dominant follicle compared to humans. Ovulation occurs spontaneously every 4 - 5 days. There are some dominant follicles will ovulate. Ovulation in mice can produce >10 oocytes from each ovary[13,14]. Oxidative stress can interfere the self-improvement mechanisms in mitochondria and trigger a prolonged autphagy process in granulosa cells, which impacts on folliculogenesis inhibition[15,16]. Various *in vivo* and *in vitro* studies have been conducted to examine the protective effect and quality of ALA as an antioxidant in diseases also improve follicular development [17]. This improvement might due to a decrease in ROS levels and an increase in total antioxidant capacity in the follicle[15].

This study results showed that the effect of ALA, IRS-1, and GLUT-4 on the variation of folliculogenesis was found highest in the number of de Graaf follicular (48.4%) followed by secondary follicle (48.2%), corpus luteum (41,9%), tertiary follicular (31,1%), and primary follicles (22.6%) while the rest is influenced confounding factor. The effect of ALA administration and lowered insulin ressistance in PCOS models demonstrated the highest effect on de Graaf follicles around 48.4%, shows there were other factors that might influence. The current perspective sees PCOS with insulin resistance as a complex disorder, in which many variants and factors combine and contribute to the pathophysiology. This complexity will complicate the therapy. However, one of the limitations in this study was the absence of control using normal rats, resulting no data on expression of IRS-1 muscle expression, ovarian expression and GLUT-4 muscle in normal model.

Conclusion

There were a significantly differences on IRS-1 expression in straited muscles and ovary, GLUT-4 in muscle cells, and de Graaf follicle number between treatment and control groups. There were no signinificant difference in the number of primary, secondary, tertiary and corpus luteum between all groups.

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Conflict of Interest: There is no conflict of interest.

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