

The Effect of Cangfudaotan Tang on Expression of Organic Anion Transporting Polypeptide (oatp4a1) in Rat Ovary and Uterus Tissues of Obese PCOS

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Abstract

Objective: The objective is to explore the effect of Cangfudaotan Tang on expression of organic anion transporting polypeptide (oatp4a1) in ovary and uterus tissues in rats with obese polycystic ovary syndrome. **Methods:** Poresky method and high fat diet were used to build obese PCOS rat models. The PCOS rat models were randomized into model control group, metformin group, the low dose Cangfudaotan Tang group and the high dose Cangfudaotan Tang group with 10 rats in each group. The metformin group was treated with metformin 43 mg/100g intragastric administration for two weeks, and the high dose Cangfudaotan Tang group and the low dose Cangfudaotan Tang group were given Cangfudaotan Tang 5.68 g/kg/d and 1.42 g/kg/d intragastric administration respectively for two weeks. Then the rats were anesthetized. Each rat was taken ovary and uterus tissues, and the expression of oatp4a1 Protein was detected in the tissues by using Western Blot techniques. The level of sex hormones as testosterone (T), follicle stimulating hormone (FSH), estradiol (E2), and luteinizing hormone (LH) and insulin resistance were detected by ELLISA techniques. **Results:** The expression of oatp4a1 in rat ovary and uterus tissue of model group was down-regulated compared to blank control group ($p \leq 0.05$). The expression of oatp4a1 in ovary and uterus tissue of treatment group was up-regulated compared to model group ($p < 0.05$). The metformin group and the high doses Cangfudaotan group were more apparent, while there was no statistical significance between the metformin group and the high dose Cangfudaotan group. Moreover, the model PCOS group had higher serum T, LH/FSH ($p < 0.01$) and lower E2 level ($p < 0.01$) compared to normal group. After treatment, serum T, LH ($p < 0.01$) and LH/FSH ($p < 0.01$) were decreased in metformin group and Cangfudaotan Tang group ($p < 0.01$) compared with those in PCOS model group. However, there was no significantly difference in serum T, LH, LH/FSH and

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E2 between metformin group and the high dose Cangfudaotan Tang group. Conclusion: The mechanism of Cangfudaotan Tang treatment of obese PCOS may be correlated to oatp4a1 by transportation and transformation of phlegmy dampness and regulating the levels of sex hormone.

Keywords

Cangfudaotan Tang, Polycysticovarian Syndrome, oatp4a1, Sex Hormone

1. Introduction

Polycystic ovary syndrome (PCOS) is an endocrine and metabolic disorder that affects approximately 6% - 10% women of reproductive age. The mechanism of PCOS is not yet clarified. It has linked to multiple systems, such as genetic, endocrine, immune and metabolic systems [1]. PCOS is regularly associated with obesity with a prevalence of 50% - 60% [2]. In previous studies, we found that Organic Anion Transporting Polypeptide might be the material basis involved in transportation and transformation of dampness. The abnormal Expression of Organic Anion Transporting Polypeptide is correlated with obese PCOS. Cangfudaotan Tang is one of prescriptions in stagnation of phlegm-dampness type of PCOS. So our objective of study is to explore the effect of Cangfudaotan Tang on expression of organic anion transporting polypeptide (oatp4a1) in ovary and uterus tissues in rats with obese PCOS. The findings of the report are as follows.

2. Methods

All experiments were carried out in strict accordance with the Guide for the Care and Use of Laboratory Animals by Sun Yat-Sen university experimental animal ethics committee. 50 female Sprague-Dawley rats (One-day old of age) weighting to 35 - 45 g were purchased from the animals central of Guangdong province. The animals were housed in a temperature-controlled ($23^{\circ}\text{C} \pm 1^{\circ}\text{C}$) with a 12 h light/dark cycle. General symptoms such as body weight, the state of activity, the quantity of diet and excrement situation were closely observed and daily vaginal smears were taken.

2.1. Production of Obese PCOS Rat Models

PCOS rat models were referenced to Luoshi modified PCOS rat models [3]. 21-day old Sprague-Dawley female rats were allowed to acclimatize for 2 days before the beginning of the experiment. 23-day old rats were injected with Sodium Prasterone Sulfate for Injection (9 mg/100g) in the back every day (40 days for a course). When the Sprague-Dawley female rats reached 27 Days of age, all rats received a single i.m. injection of human chorionic gonadotropin (HCG) at dose of 1.5 units of HCG (35 days for a course). High fat diet was used to build obese PCOS rat models.

2.2. Animal Grouping and Intervention

21-day old Sprague-Dawley female rats weighting to 35 - 45 g were randomly divided into 4 groups: normal group (n = 10), blank model group (n = 10), metformin group (n = 10), the low does Cangfudaotan group (n = 10), the high does Cangfudaotan group (n = 10).

After obese PCOS Rat Models were made, the metformin group was treated with metformin 43 mg/100g intragastric administration for two weeks. The Cangfudaotan group was administration with Cangfudaotan that comes from the recipe of treatment of Tianshi Ye. Cangfudaotan Tang can transport and transform of dampness. It includes Rhizoma atractylodis 10 g, Rhizoma cyperi 10 g, Fructus Ponciri 10 g, fabanxia 10 g, *Pericarpium citri nobilis* 6 g, Poria 15 g, Arisaematis Rhizoma 6 g, Radix glycyrrhizae 6 g, Zingiberis Rhizoma 6 g. We boiled them into concentrated liquor according to specific technological process. Cangfudaotan Tang was stored at $2^{\circ}\text{C} - 8^{\circ}\text{C}$ for preservation. The Cangfudaotan Tang high and low dose groups were given Cangfudaotan Tang 5.68 g/kg/d and 1.42 g/kg/d intragastric administration respectively for two weeks. The normal group was treated with free diet and drinking water, intragastrically administrated by 0.9% saline for 2 weeks.

2.3. Endometrium and Ovarian Morphology Assessment

The rats were sacrificed at 78-day of age. The rats were anesthetized with a fixative of 10% pentobarbital sodium solution. The ovary and uterus were cleaned of adherent fat tissue and immediately frozen in liquid nitrogen at -80°C . To assess morphology endometrium and ovarian, the samples were fixed in 4% formaldehyde buffer for 24 hours, dehydrated and embedded in paraffin. Ten microns-thick serial histological sections were made and stained with hematoxylin-eosin. All the sections were analyzed for the presence of corpora lutea (CL), healthy antral follicles and follicular cysts with the aid of a Nikon binocular microscope.

2.4. Hormone Measurement

The blood from the heart was collected, allowed to clot, and centrifuged during 15 min at 3000 RPM. The serum was stored at -20°C , until T, E2, FSH and LH levels were measured. LH (mIU/ml), FSH (mIU/ml), T (ng/ml) and E2 (pg/ml) serum concentrations were measured using ELISA, with kits were purchased from Guangzhou yale pharmaceutical company.

2.5. Statistical Analyses

The results were expressed as mean \pm standard error of mean. The number of subjects was calculated with $\alpha = 0.05$. The distribution of the data was analysed by the Kolmogorov-Smirnov test. The results were analysed by Student's t-test or Mann-Whitney test. The statistical differences were considered with p-values less than 0.05. Probability values below 0.05 were considered statistically significant.

3. Result

3.1. The Expression of oatp4a1 in Rat Ovary and Uterus Tissue

Table 1 showed that compared with blank control group, model group was down-regulated ($p < 0.01$). The expression of oatp4a1 in ovary and uterus tissue of treatment group was up-regulated compared to model group ($p < 0.05$), the metformin group and the high does Cangfudaotan group were more apparent, while there was no statistical significance between the metformin group and the high dose Cangfudaotan group ($p > 0.05$) (**Figure 1**).

3.2. The Distribution of oatp4a1 in Rat Ovary and Uterus Tissue

Immunohistochemical assay showed that the expression of oatp4a1 was observed in glandular epithelium and cells infiltrating the stroma (see **Figure 2**). H&E staining showed an increase in the number of glands, an expansion of glandular cavity and many wrinkles in blank control group. There is a decreased in the number of glands, a shrink of glandular cavity and no apparent wrinkle in model group The number of glands of treatment group was up-regulated compared to model group ($p < 0.05$). The glandular cavity became small and straight and

Table 1. The expression of oatp4a1 in rat ovary and uterus tissue.

group	blank	model	low CFDTT	high CFDTT	metformin
oatp4a1 (uterus)	0.53 ± 0.32	$0.12 \pm 0.04^{##}$	$0.32 \pm 0.12^*$	$0.55 \pm 0.35^{**}$	$0.54 \pm 0.36^{**}$
oatp4a1 (ovary)	0.61 ± 0.19	$0.24 \pm 0.03^{##}$	$0.35 \pm 0.26^{*#}$	$0.62 \pm 0.15^*$	$0.61 \pm 0.34^*$

Compared with blank control group, [#]Correlation is significant at the 0.05 level. ^{##}Correlation is significant at the 0.01 level. Compared with model group, ^{*}Correlation is significant at the 0.05 level. ^{**}Correlation is significant at the 0.01 level.

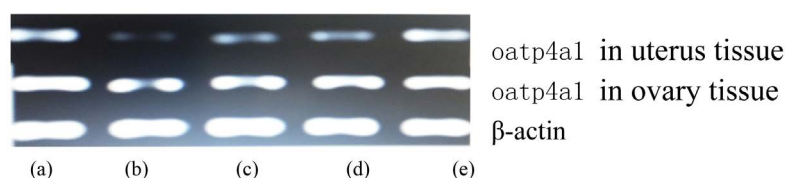


Figure 1. The expression of oatp4a1 in ovary and uterus tissue by Western blotting: (a) blank control group; (b) model group; (c) the low does Cangfudaotan group; (d) the high does Cangfudaotan group; (e) metformin group.

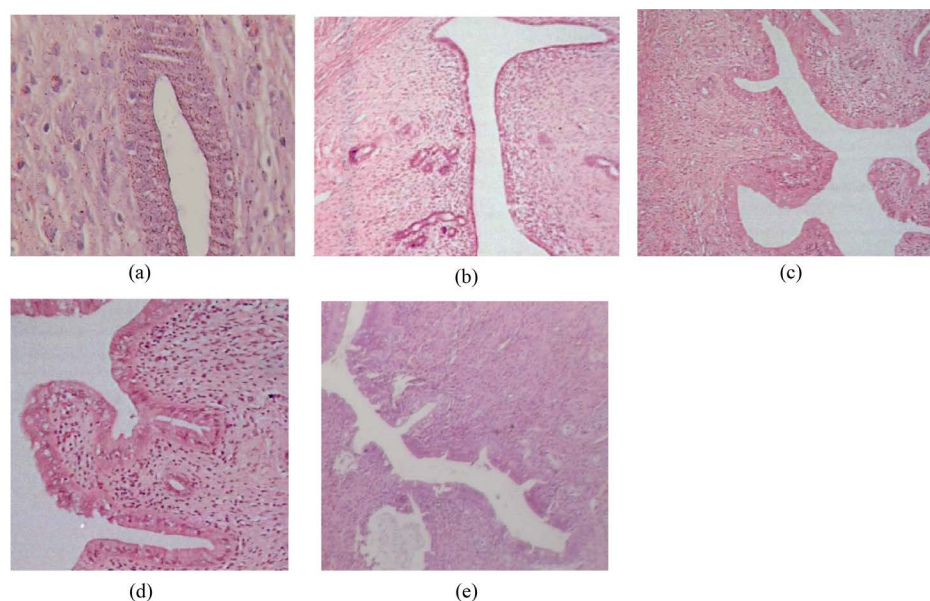


Figure 2. The expression of oatp4a1 in endometrium tissue by immunohistochemistry (HE*250): (a) blank control group; (b) model group; (c) the low does Cangfudaotan group; (d) the high does Cangfudaotan group; (e) metformin group.

appeared wrinkles in treatment group. The expression of oatp4a1 of treatment group was up-regulated compared to model group ($p < 0.05$), the metformin group and the high does Cangfudaotan group were more apparent, while there was no statistical significance between the metformin group and the high dose Cangfudaotan group.

H&E staining showed the ovaries in the blank control group exhibited follicles at different stages and the presence of corpora lutea (**Figure 3**). The ovaries in the model group displayed follicular cysts with a diminished granulosa cell compartment that was arranged loosely. The expression of oatp4a1 of the ovaries was down-regulated ($p < 0.05$). Treatment group displayed some well-developed follicles. The expression of oatp4a1 of the metformin group and the high does Cangfudaotan group was up-regulated compared to model group ($p < 0.05$).

3.3. Hormones Levels

Table 2 showed that the model PCOS group had higher serum T, LH/FSH ($p < 0.01$) and lower E2 level ($p < 0.01$) compared to blank control group. After treatment, serum T, LH ($p < 0.01$) and LH/FSH ($p < 0.01$) were decreased in metformin group and Cangfudaotan Tang group ($p < 0.01$) compared with those in PCOS model group. However, there was no significantly difference in serum T, LH, LH/FSH and E2 between metformin group and the high dose Cangfudaotan Tang group.

4. Discussion

Polycystic ovary syndrome (PCOS) belongs to the disease of menstruation in Chinese Medicine. The theory of Chinese medicine found: 1) spleen is the source of phlegm; 2) Eating too much something fat will cause spleen deficiency generate phlegm; 3) The cause of the obese PCOS is Phlegmy. Stagnation of phlegm-dampness is an important type of the obese PCOS in Chinese medicine. It usually invigorates spleen to remove phlegm. Cangfudaotan Tang is one of prescriptions in stagnation of phlegm-dampness type of PCOS. Organic Anion Transporting Polypeptide might be the material basis involved in transportation and transformation of dampness. However, the molecular biology mechanism of invigorating spleen to remove phlegm was unclear.

Organic anion transfer peptide (oatp) is an important membrane transporter in animal's body which works as uptake transporters of a wide variety of drugs, xenobiotics and endogenous substances [4] [5]. In previous studies [6]-[8], we have described that oatps can efficiently transfer a variety of foreign organisms and play a key role in transit dampness. And here was a relationship between oatps and PCOS. Plaza described for the first time the expression of transporters OATP-B, D and E in human endometrium. The expression of OATP-E is associated

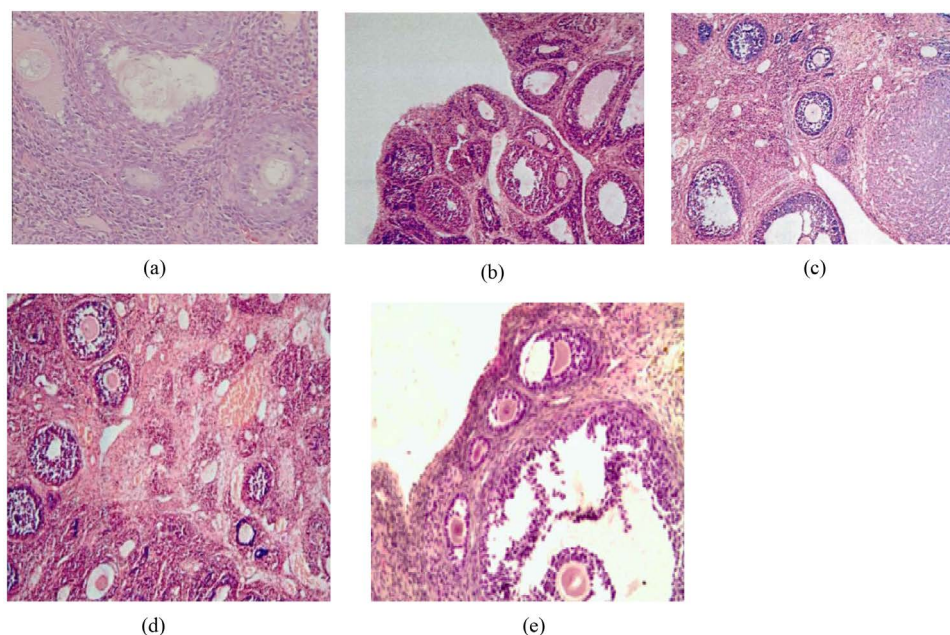


Figure 3. The expression of oatp4a1 in ovarian tissue by immunohistochemistry (HE*250): (a) blank control group; (b) model group; (c) the low does Cangfudaotan group; (d) the high does Cangfudaotan group; (e) metformin group.

Table 2. Endocrine characteristics ($\bar{x} \pm s$, n = 10).

group	LH (mIU/ml)	FSH (mIU/ml)	LH/FSH	T (ng/ml)	E2 (pg/ml)
blank	23.67 ± 4.62	8.69 ± 3.43	1.94 ± 2.42	0.79 ± 0.23	23.36 ± 3.51
model	48.71 ± 5.54 ^{##}	7.94 ± 2.53 [#]	5.09 ± 2.17 ^{###}	1.72 ± 0.69 ^{###}	11.24 ± 3.94 ^{##}
low CFDTT	26.32 ± 6.02 [*]	8.03 ± 3.71	3.12 ± 1.66 [#]	0.47 ± 0.26 [#]	23.81 ± 4.19 [*]
high CFDTT	21.41 ± 3.58 ^{**} [△]	9.75 ± 3.55 [*]	2.24 ± 1.32 [*]	0.21 ± 0.28 ^{**}	22.76 ± 2.85 ^{**}
metformin	24.34 ± 3.61 ^{**}	9.18 ± 2.62 [*]	2.32 ± 1.19 ^{**}	0.24 ± 0.31 ^{**}	21.41 ± 2.73 ^{**}

Compared with blank control group, [#]Correlation is significant at the 0.05 level. ^{##}Correlation is significant at the 0.01 level. Compared with model group, ^{*}Correlation is significant at the 0.05 level. ^{**}Correlation is significant at the 0.01 level. Compared with metformin group, [△]Correlation is significant at the 0.05 level.

with the menstrual cycle. The OATP-E transporter transcript levels were the only one increased in the control endometrium in the secretory phase of the menstrual cycle. On the other hand, a lower transcript expression of OATP-E in the endometrium of PCOS women was observed compared with the control group. As mentioned, the DHEA-S requires OATP-E to enter cells. The high plasma concentration of DHEA-S, coupled with the high levels of the transporter OATP-E, suggest a potential higher uptake of DHEA-S into the endometrial cells from PCOS women. Sai Y. found that OATP-B was expressed in the apical membranes of Caco-2 cells. The results suggested that intestinal absorption of estrogen in humans is likely to be mediated by OATP-B [9]. Therefore, oatp is associated with the secretion, metabolism and absorption of endocrine hormone in the body, contributing to the occurrence of PCOS.

In this study, the expression of oatp4a1 in rat ovary and uterus tissue of model group was down-regulated compared to blank control group. The expression of oatp4a1 in ovary and uterus tissue of treatment group was up-regulated. The high does Cangfudaotan group were more apparent, while there was no statistical significance between the metformin group and the high dose Cangfudaotan group.

5. Conclusion

In conclusion, stagnation of phlegm-dampness is an important type of the obese PCOS in Chinese medicine. The disorder of transiting dampness plays an important role in PCOS. The expression of oatp4a1 in rat ovary and

uterus tissue of model group was down-regulated. Cangfudaotang Tang will improve the expression of oatp4a1 in PCOS, which suggested the enhanced ability in transporting dampness. The mechanism of Cangfudaotan Tang treatment of obese PCOS may be correlated to oatp4a1 by transportation and transformation of phlegmy dampness and regulating the levels of sex hormone.

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