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Effects of dipeptidyl peptidase-4 inhibitors on transforming growth factor-β1 signal transduction pathways in the ovarian fibrosis of polycystic ovary syndrome rats

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Abstract

Aim: Examine the effects of dipeptidyl peptidase-4 (DPP4) inhibitor Sitagliptin on the transforming growth factor-β1 (TGF-β1) signal transduction pathway in polycystic ovary syndrome (PCOS) rats with ovarian fibrosis.

Methods: Thirty rats were divided randomly into the PCOS model group, Sitagliptin treatment group and blank control group. Dehydroepiandrosterone was administered to the model group and treatment group to establish the models. Then, the phenotype of rats was recorded, and the serum sex hormone levels were measured. The pathological structures of the rat ovaries were observed. The protein and mRNA expression levels of DPP4, connective tissue growth factor (CTGF), TGF-β1 and Smad2/3 in the ovaries were analyzed.

Results: There was no statistically difference in fasting body weight and blood glucose among the three groups before Sitagliptin treatment ($P > 0.05$). The fasting blood glucose level was significantly decreased after the administration of Sitagliptin ($P < 0.05$). The level of testosterone in the model group was reduced remarkably after Sitagliptin treatment ($P < 0.001$). The protein expression levels of DPP4, CTGF and TGF-β1 in the ovarian stroma were lower in the treatment group than in the model group ($P < 0.01$, $P < 0.001$, $P < 0.05$). The mRNA levels of DPP4, CTGF and TGF-β1 in the model group also greatly declined after Sitagliptin treatment ($P < 0.05$, $P < 0.001$, $P < 0.01$).

Conclusion: The DPP4 inhibitor Sitagliptin lowers fasting blood glucose, relieves the high androgen state of PCOS rats and delays the process of ovarian fibrosis, which may be related to reducing the levels of factors related to the TGF-β1/Smad2/3 signaling pathway.

Key words: endocrine, infertility, ovarian function, polycystic ovary syndrome.

Introduction

Polycystic ovary syndrome (PCOS) is characterized by clinical manifestations such as ovulation failure and insulin resistance. One of the mechanisms of ovulatory dysfunction is ovarian stromal fibrosis. The TGF-β1/Smad2/3 pathway is an important signaling pathway for tissue fibrosis, playing a significant role in the fibrotic diseases of various organ and tissue types. Researchers have confirmed that fibrosis is closely related to the synthesis of extracellular matrix (ECM) components. Many scholars have suggested this Signal pathways may be the key of tissue fibrosis.[1] Connective tissue growth factor (CTGF) is the downstream reaction element of transforming growth factor-β1 (TGF-β1) and can induce fibroblast proliferation and become involved in the formation of the ECM. Dipeptidyl peptidase-4 (DPP4) inhibitor has

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been used to cure insulin resistance (IR) for a long time, and in recent years, DPP4 has been reported to work against fibrosis and inflammation. DPP4 can mitigate the fibrosis of viscus and organs, such as myocardium, liver, kidney and lung.[2–5] However, there have not been any related reports on ovarian fibrosis. This research aimed to examine the effects of DPP4 inhibitor Sitagliptin on the PCOS rat model of ovarian fibrosis and evaluate the associated mechanism.

Methods

Animals

Thirty aseptic female Sprague–Dawley rats were used in this study. Rats were maintained according to the Guide for the Care and Use of Laboratory Animals. These 21-day-old rats, weighing 50–60 g, were purchased from the Animal Center of Zhejiang Academy of Medical Sciences. Other reagents were purchased as follows: Sitagliptin (Januvia) (Merck Sharp Dohme Ltd.), dehydroepiandrosterone (DHEA) (China Pharmaceutical Group Chemical Reagent Co. Ltd.) and injection-oriented camellia oil (Zhejiang Tianyushan Medicinal Oil Co. Ltd.).

Treatment

Thirty 21-day-old rats were fed standard forage and allowed to eat and drink freely. The rats were fed for 3 days, and the 24-day-old rats were randomly divided into the control group, model group and treatment group (10 rats per group). DHEA was dissolved in injection-oriented camellia oil; 0.2 mL of DHEA was injected at 6 mg/100 g daily subcutaneously in to the model group and treatment group, and the same amount of camellia oil was injected in to the control group. Vaginal smears were obtained from all rats beginning on the 11th day, and hypodermic injections and vaginal smears stopped being performed on the 20th day. Later, the treatment group was given 2 mL of Sitagliptin at 63 mg/100 g daily by gavage, while the control group and model group were given the same amount of distilled water for 28 consecutive days. Vaginal smears were obtained from all rats during the last 10 days.

After taking medications, all rats were weighed on an empty stomach; fasting blood glucose levels were measured in the morning; chloral hydrate (**3.**5 μL/g) was injected into the abdominal cavity to anesthetize the rats, and inferior blood was extracted from the postcava. Serum was collected after centrifugation and stored in a freezer (−80°C) for subsequent ELISA to measure the levels of testosterone (T), luteinizing hormone (LH), follicle-stimulating hormone (FSH), the LH/FSH ratio and anti-Mullerian hormone (AMH). The ovaries were weighed, and one was placed in 10% neutral formalin. The largest plane of the ovary was taken as the inspection plane for paraffin embedding, cut into slices continuously (4 μm thick) and pasted onto poly-L-lysine clean slices to prepare sections. Some were used for HE staining to observe the ovarian pathological structure, and some were used for immunohistochemical experiments after baking. The other ovary was frozen in liquid nitrogen for storage at −80°C (half for Western blot and half for PCR).

Immunohistochemistry

The expression levels of the DPP4, TGF-β1, Smad2/3 and CTGF in the ovarian stroma were tested. This study employed the streptomycin-biotin peroxidase immunohistochemical staining method (S-P Method). The primary antibodies were all goat anti-rabbit polyclonal antibodies, and the secondary antibodies were all goat anti-rat secondary antibodies. Counterstaining with hematoxylin and coloration with diaminobenzidine were performed. Analysis was carried out with the semiquantitative integral method. Ten fields of vision at high magnification were chosen randomly for every section to analyze the positive cell number and coloration strength. Scores were assigned according to the positive cell percentage as follows:0 points for a positive cell percentage <6%, 1 point for a positive cell percentage from 6 to 25%, 2 points for a positive cell percentage from 26 to 50%, 3 points for a positive cell percentage from 51 to 75% and 4 points for a positive cell percentage >75%. Scores were assigned according to coloration strength as follows: 0 points for no staining, 1 point for light yellow staining, 2 points for yellow staining and 3 points for tan staining. The total score was calculated by multiplying the two groups of scores, and 0 points were classified as negative (–), 1–4 points were classified as weakly positive (+), 5–8 points were classified as positive (++) and 9–12 points were classified as strongly positive (+++).

Western blot analysis

The DPP4, TGF-β1, Smad2/3 and CTGF protein levels in the ovarian tissues were tested. The films were scanned, archived and decolorized by PhotoShop.

The optical density value of the target band was analyzed with an Alpha software analysis system, and the ratio of the target and gray level to the internal reference band gray level was calculated.

Real-time quantitative polymerase chain reaction analysis

The mRNA levels of DPP4, TGF-β1, Smad2/3 and CTGF in the ovarian tissues were analyzed. Total RNA was extracted with the Trizol method, and RNA concentrations and purity were tested with a Nanodrop 2000 spectrophotometer. RNA was reversed transcribed into cDNA according to the instructions provided in the reverse transcriptase kit and amplified in accordance with the instructions provide in the PCR kit. The general reaction volume was 40 μL, and the PCR conditions were as follows: denaturation at 95°C for 10 min; 40 cycles of 60°C → 95°C for a melting curve and increases by increments of 1°C every 20 s. The ΔΔCT method was used to analyze the results. The primer manufacturer was Service Bio, and the specific primers were as follows: R-DPP4-S primer sequence AACCCCACTTCACCTCCGAC and R-DPP4-A primer sequence GACCTGTTCGGGTTTC CTATCT, segment length 107 bp; R-Smad2-S primer sequence ACTGCCGCCTCTGGATGACTAT and R-Smad2-A primer sequence AGAGAGTGGTAGGA-GACAGTTCAGC, segment length 197 bp; R-Smad3-S primer sequence CGAGAACACTAACTTCCCCGCT and R-Smad3-A primer sequence GTGGTTCATCTGG TGGTCGCTA, segment length 112 bp; R-TGF-β1-S primer sequence CTTTAGGAAGGACCTGGGTTG and R-TGF-β1-A primer sequence GGTTGTGTTGGTT GTAGAGGG, segment length 140 bp; R-Ctgf-S primer sequence CCAACTATGATGCGAGCCAACT and R-Ctgf-A primer sequence TTAGCCCGGTAGGT CTTCACACT, segment length 272 bp.

Statistical analyses

Stata 14.0 was used for the analysis of the experimental data. The data were not in conformity with the test of normality and homoscedasticity. Thus, statistical analysis was carried out using a nonparametric test, quantitative materials were described as the interquartile range M (Q25, Q75), and the rank-sum test was used to make comparisons between groups. The Kruskal–Wallis test was conducted for ranked data to make comparisons between groups. $P < 0.05$ indicated a statistically significant difference.

Results

Phenotypes

There were no statistically significant differences in fasting body weight or blood glucose among the three groups before Sitagliptin treatment ($P > 0.05$, $P > 0.05$). After Sitagliptin treatment, the fasting blood glucose level in the treatment group was significantly lower than that in the model group ($P < 0.05$), but there were no significant differences in weight among the three groups ($P > 0.05$). Changes in the estrus cycles of the three groups of rats were also evaluated. After the establishment of the PCOS model and before Sitagliptin treatment, the rats in the blank control group had regular estrus cycles. The other two groups had no normal estrus cycles. Though there was still no regular estrus cycle in the treatment group, pre-estrus were the main stages observed, while, dioestrus mainly for model group.

Evaluation of models

After vaginal smears were collected for 10 days continuously, regular estrous cycles were observed in all rats of the control group, while 90% of the PCOS group did not have normal estrous cycles; thus, the PCOS models were built successfully for the preliminary assessment. A morphological comparison was made by HE staining of rat ovarian tissues after all treatments (Fig. 1). In the control group, the ovaries were in good condition, and the follicles and corpora lutea of the different development all stages were visible (Fig. 1a). In the PCOS model group, the ovarian volume was unusually increased, and there were a large number of unusually enlarged follicles within the ovaries. The theca cells and cellular layers of the follicles were thickened, the granular cell layers had become thinner and granular cell placement was chaotic. Some of the follicles underwent vacuolar degeneration and necrosis. A large amount of ovarian follicular fluid filled the follicles, and leukomonocytes were visible in a small amount of ovarian follicular fluid (Fig. 1b). The PCOS rat models were built successfully for further evaluation. Additionally, the Sitagliptin treatment group showed a regular ovarian structure, fewer unusually increased follicles, no obvious increase in theca cell layers of follicular cells and an organized arrangement of granular cells (Fig. 1c).

Comparison of serum hormone levels

The concentrations of T and AMH in the serum increased in the PCOS model group ($P < 0.001$, $P < 0.01$) compared with the control group, but the

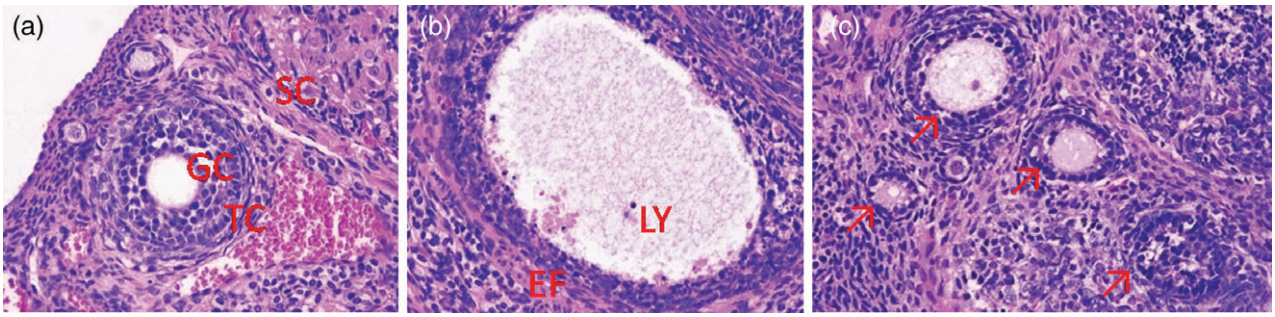

Figure 1 Morphological of ovarian cells among groups' rats HE staining (hematoxylin and eosin [H&E] × 400): (a) blank control group, (b) Polycystic ovary syndrome (PCOS) model group, (c) Sitagliptin treatment group; GC granular cell, TC theca cell, SC stromal cell, EF excessively enlarged follicle, LY lymphocyte, ↗ each stage follicles.

LH/FSH ratio was not statistically significant ($P > 0.05$). The levels of T in the Sitagliptin treatment group were reduced compared with those in the PCOS model group ($P < 0.01$), but the differences in AMH and LH/FSH were not statistically significant between the treatment and model groups ($P > 0.05$) (Fig. 2).

Protein expression levels of DPP4, TGF-β1, Smad2/3 and CTGF in the ovary

Comparison of the protein levels of DPP4, TGF-β1, Smad2/3 and CTGF in the whole ovary

The protein levels of DPP4 in the PCOS model group rose noticeably ($P < 0.001$) compared with those in the control group, and the levels of TGF-β1 and CTGF increased ($P < 0.01$). The protein levels of TGF-β1 were reduced in the Sitagliptin treatment group ($P < 0.001$), and those of DPP4 and CTGF were decreased ($P < 0.01$). There was no statistical significance regarding Smad2 or Smad3 among the three groups ($P > 0.05$) (Fig. 3).

Protein expression levels of DPP4, TGF-β1, Smad2/3 and CTGF in the ovarian stroma

The DPP4, TGF-β1, Smad2/3 and CTGF proteins were expressed in the ovarian stroma of rats. A comparison of the PCOS model group with the treatment group showed that the expression levels of DPP4, CTGF, TGF-β1, Smad2 and Smad3 were all increased in the ovarian stroma of the rats, with notable differences for TGF-β1, Smad2 and Smad3 ($P < 0.001$). A comparison of the Sitagliptin treatment group with the PCOS model group showed that the protein expression intensity of DPP4, TGF-β1 and Smad3 was notably decreased ($P < 0.001$), but this decrease was not observed for Smad2 or CTGF (Table 1) (Fig. 4).

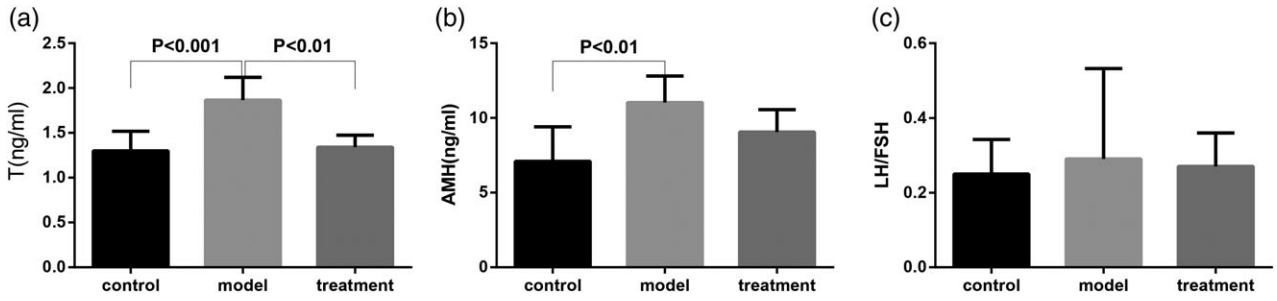

Figure 2 ELISA method was employed to test the testosterone (T), anti-Mullerian hormone (AMH), luteinizing hormone/follicle-stimulating hormone (LH/FSH) levels of each group. Control represented the blank control group, model represented polycystic ovary syndrome (PCOS) model group, treatment represented Sitagliptin treatment group. The data was analyzed and described by interquartile range: (a) Comparison of T level (ng/mL): the level of the treatment group 1.34 (1.30, 1.43) ng/mL was below of that of the model group 1.47 (1.86, 2.11) ng/mL greatly ($P < 0.01$); (b) Comparison of AMH level (ng/mL): there was no statistical significance between the levels of treatment and the model group, while the level of the model group 11.04 (8.81, 12.69) ng/mL was above of the control group 7.09 (4.96, 9.38) ng/mL noticeably ($P < 0.01$); (c) Comparison of ratio of LH/FSH: there was no statistical significance among the three groups. Control ■, model ■, treatment ■

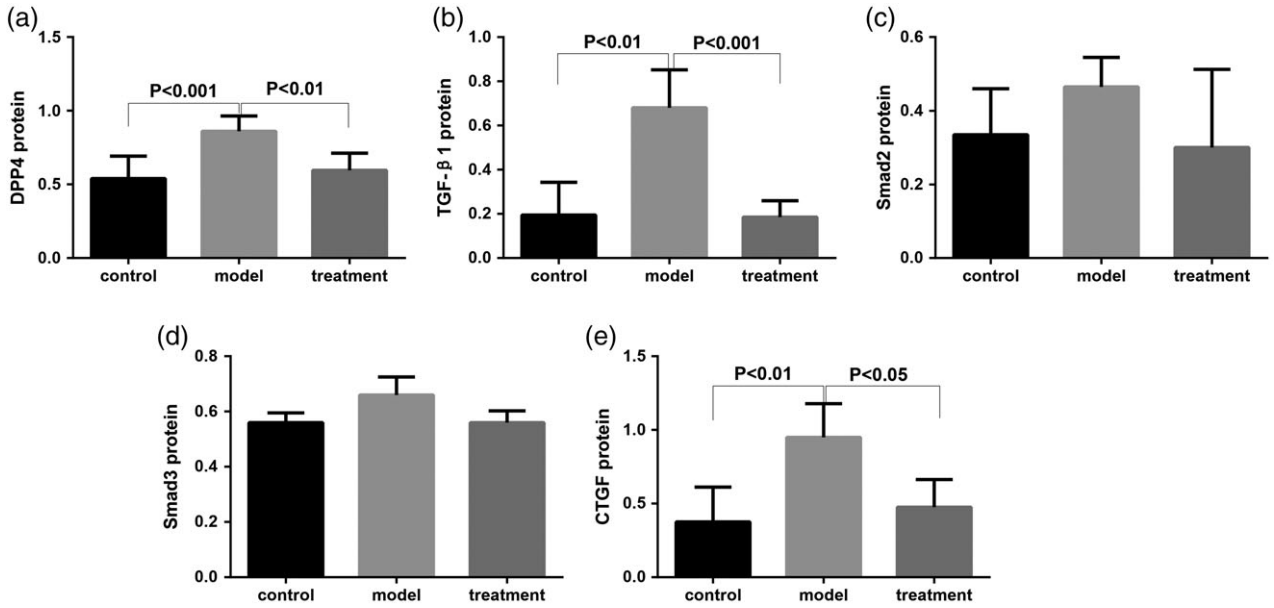

Figure 3 Western blot method was used to analyze the ratio of objective band gray level to internal reference band gray level of the dipeptidyl peptidase-4 (DPP4), transforming growth factor-β1 (TGF-β1), Smad2, Smad3 and connective tissue growth factor (CTGF) proteins. The data was analyzed and described by the interquartile range M (Q25, Q75). (a) DPP4: the level of the model group 0.86 (0.72, 0.94) rose notably ($P < 0.001$) compared with that of the control group 0.54 (0.46, 0.69), while the level of the treatment group 0.60 (0.46, 0.71) reduced greatly ($P < 0.01$) compared with that of the model group; (b) TGF-β1: the level of the model group 0.68 (0.35, 0.82) increased notably ($P < 0.01$) compared with the control group 0.20 (0.10, 0.33), while the level of the treatment group 0.19 (0.14, 0.24) dropped greatly ($P < 0.001$) compared with the model group; (e) CTGF: the level of the model group 0.95 (0.78, 1.15) increased notably ($P < 0.01$) compared with that of the control group 0.37 (0.21, 0.59), while the level of the treatment group 0.48 (0.35, 0.61) reduced greatly ($P < 0.05$) compared with that of the model group; (c) Smad2; (d) Smad3: the difference among the three groups had no statistical significance. Control ■, model ■, treatment ■

Table 1 Proteins' expressions of DPP4, TGF-β1, Smad2/3 and CTGF in ovary stroma

Intensity	DPP4				TGF-β1				Smad2				Smad3				CTGF			
	−	+	++	+++	−	+	++	+++	−	+	++	+++	−	+	++	+++	−	+	++	+++
Control group	2	0	6	2	0	8	2	0	−	8	2	0	0	10	0	0	−	10	0	0
Model group	0	0	2	8	0	0	4	6	−	0	6	4	0	0	6	4	−	4	4	2
Treatment group	2	0	8	0	4	4	2	0	−	0	8	2	2	6	2	0	−	8	0	2

(1) Scoring according to positive cell percentage:0 point for positive cells number, 1 point for 6–25%, 2 points for 26–50%, 3 points for 51–75%, 4 points for positive cells number >75%; (2) Scoring according to coloration strength: 0 point for nonstaining, 1 point for light yellow, 2 points for yellow and 3 points for tan. Multiply the two group of scores as the total score, and categorize 0 point as negative (−), 1–4 points as weakly positive (+), 5–8 points as positive (++), 9–12 ≤5% points as strongly positive (+++). DPP4, dipeptidyl peptidase-4; TGF-β1, transforming growth factor-β1; CTGF, connective tissue growth factor.

mRNA expression levels of DPP4, CTGF, TGF-β1 and Smad2/3 in the ovaries

In comparisons involving the $2^{-\Delta\Delta Ct}$ value, the mRNA level of DPP4 in the PCOS model increased notably compared with that in the control group ($P < 0.001$) and increased for TGF-β1 and CTGF ($P < 0.05$), while the mRNA levels of DPP4, TGF-β1 and CTGF in the Sitagliptin treatment group decreased compared with those in the PCOS model group ($P < 0.05$). The differences in the mRNA levels of Smad2 and Smad3 were not statistically significant among the three groups ($P > 0.05$) (Fig. 5).

Discussion

PCOS patients have a difficulty in pregnancy. The primary cause of this difficulty is ovulatory dysfunction,

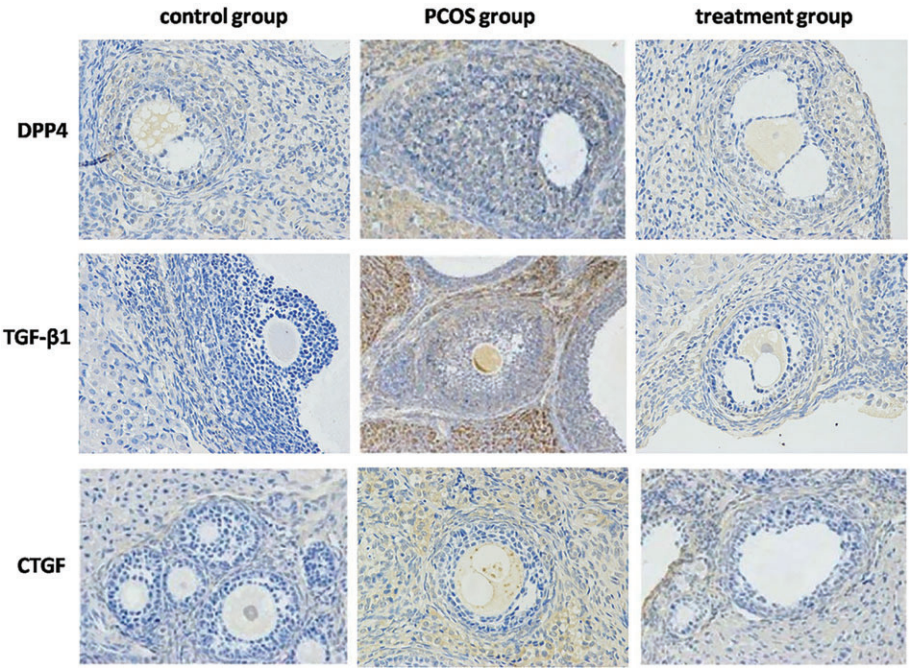

Figure 4 Proteins' expressions of dipeptidyl peptidase-4 (DPP4), connective tissue growth factor (CTGF) and transforming growth factor-β1 (TGF-β1) in ovarian stroma for each group (×400): the expressions of DPP4, CTGF and TGF-β1 in ovarian stroma of the polycystic ovary syndrome (PCOS) group were strongly positive, while they were noticeably weaker in both the treatment group and the control group.

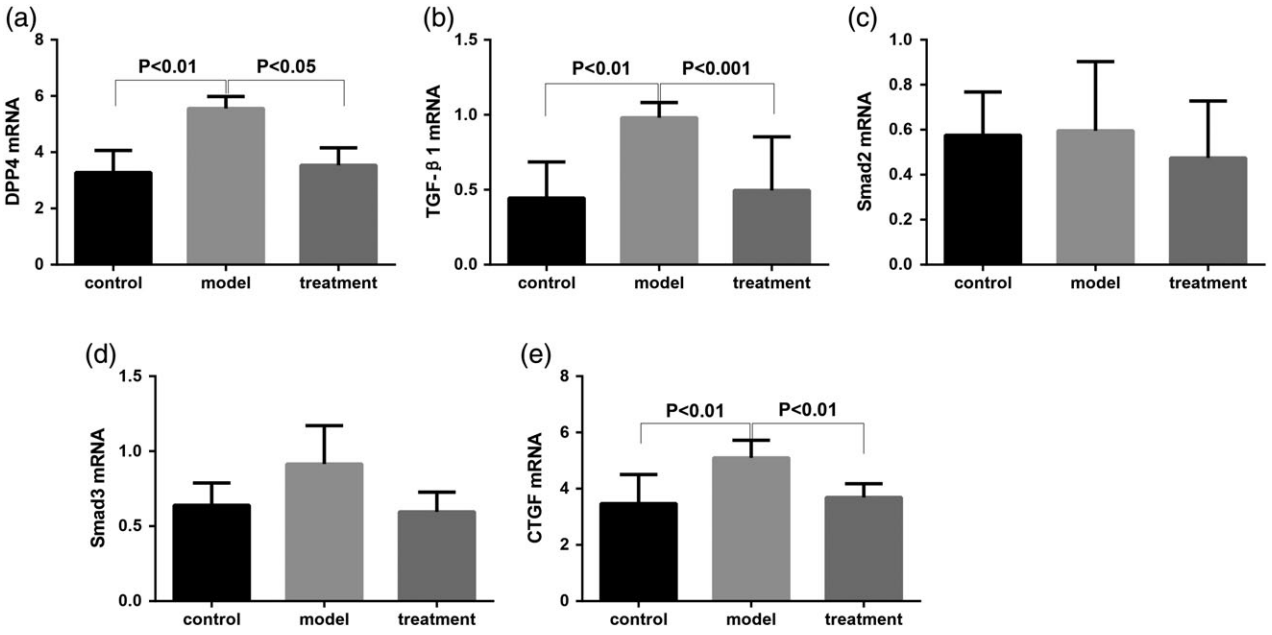

Figure 5 RT-PCR was conducted to test and analyze dipeptidyl peptidase-4 (DPP4), transforming growth factor-β1 (TGF-β1) and connective tissue growth factor (CTGF) mRNAs' $^{2-\Delta\Delta}$Ct: the data was analyzed and described by interquartile range M (Q25, Q75). (a) DPP4: the data of the model group 5.55 (4.80, 5.78) increased notably compared with that of the control group 3.28 (3.02, 3.91) ($P < 0.01$), while that of the treatment group 3.54 (3.26, 4.12) decreased compared with that of the model group ($P < 0.05$); (b) TGF-β1: the data of the model group 0.98 (0.80, 0.98) increased notably compared with that of the control group 0.45 (0.39, 0.65) ($P < 0.01$), while that of the treatment group 0.49 (0.32, 0.85) greatly decreased compared with that of the model group ($P < 0.001$); (e) CTGF: the data of the model group 5.10 (4.14, 5.57) notably increased compared with that of the control group 3.47 (2.71, 4.26) ($P < 0.01$), while that of the treatment group 3.69 (3.08, 4.14) decreased ($P < 0.05$) compared with that of the model group; (c) Smad2; (d) Smad3: there was no statistical significance among the three groups. Control ■, model ■, treatment ■

and one of the mechanisms of ovulatory failure is ovarian fibrosis, which mainly occurs in the stroma. Currently, there are no effective treatment measures for reducing ovarian fibrosis in PCOS patients. DPP4 is also called adenosine deaminase binding protein or CD26. DPP4 was first discovered in 1963 and belongs to the S9 proteasefamily.[5] As a multifunctional protein, DPP4 is involved in different biological processes, including inflammation, aggressive transformation and tumor immunity.[6,7] DPP4 inhibitors have long been applied to treat islet resistance. In recent years, there have been reports on its antifibrosis effects. DPP4 is also expressed in many types of cells, such as epithelial cells, endothelial cells, marrow cells, adipose cells, skeletal muscle cells and vascular smooth muscle cells.[8–13] Sitagliptin, one of the most common medicines of DPP4 inhibitors, is mainly used for therapy in patients with diabetes who are metformin insensitive. Okura et al.[14] suggested that DPP4 had anti-inflammatory and antioxidant effects and could mitigate the hepatic fibrosis levels of patients with cirrhosis. Researchers have shown that the antifibrosis effects of Sitagliptin may inhibit the TGF-β1/Smad2/3 signal transduction pathway to activate hematopoietic stem cells and inhibit collagen synthesis, thus influencing the fibrosis process.[15,16] TGF-β1 is closely related to tissue fibrosis,[17] promoting tissue fibrosis by increasing the levels of ECM components.[18] The TGF-β1/Smad2/3 pathway is an important signaling pathway for tissue fibrosis. Research by Inagaki and Okazaki[19] showed that hepatic fibrosis was closely related to the TGF-β1/Smad signaling pathway. However, there have not been any studies on whether this pathway is related to ovarian fibrosis in PCOS. Researchers have also shown that the TGF-β1 level in the serum of PCOS patients is relatively higher than that in the serum of normal patients.[20,21] The ovarian fibrosis of PCOS patients is suggested to be potentially related to increases in TGF-β1 levels.

In our study, the serum levels of T and AMH in the PCOS rats were relatively high, which was possibly related to DHEA-induced PCOS. Although Sitagliptin could not reduce the LH/FSH ratio, Sitagliptin lowered the T level to some degree. The decrease in T very likely played a role in the improved endocrine function of the PCOS rats, which was consistent with previous reports. The reductions in the levels of T and AMH in the PCOS rats by the DPP4 inhibitor are presumably related to several mechanisms. (i) Miao found that the follicle-mesenchymal cells in the ovaries of PCOS patients were rich in smooth endoplasmic reticulum, lipid droplets and mitochondria, forming typical steroid hormone cells, which indicated that the ability to synthesize androgens in theca-interstitial cells was higher for PCOS patients than normal. Additionally, the expression of TGF-β1 in theca-interstitial cells in PCOS patients was significantly higher than normal.[22]This finding suggested that the high expression of TGF-β1 might be related to the occurrence of hyperandrogenism. DPP4 inhibitors reduce excessive DPP4 and might mitigate ovarian tissue fibrosis and reduce androgen levels indirectly. (ii) IR was present in the majority of PCOS patients, and high insulin could directly stimulus follicles, causing secrete excessive androgen, promoting follicle enlarger and increasing the amount of sinus follicular. AMH is mainly secreted by the granular cells of the small sinus follicle, and IR leads to an increase in AMH indirectly. The DPP4 inhibitor was found to be useful for regulating the proliferation and apoptosis of beta cells in islets, inhibiting the secretion of glucagon and the formation of endogenous glucose and the DPP4 inhibitor has the positive effect of reducing blood sugar and improving islet function.[23]Therefore, alleviating IR in PCOS patients can indirectly reduce T and AMH levels.

In this study, the effect of the DPP4 inhibitor Siglitatin on the weight of PCOS rats was not obvious, which may be related to the short time of administration or the small number of cases. However, we found that this drug had some effect on the estrus cycle of PCOS rats and could push estrus from diestrus into pre-estrus. Is it possible to induce normal estrus cycles in PCOS rats after long-term administration? The reason may be related to the reduction in T and AMH by the DPP4 inhibitor. This problem needs further consideration and additional studies.

The results of this research showed that the ovarian morphology of the treatment group had been improved, with a relatively smaller number of accumulating granular cells and a regular arrangement, which might be related to the anti-inflammatory effect of Sitagliptin.[24] In addition, we found that DPP4 could be expressed in the fibroblasts, fiber cells, theca cells and granular cells of rat ovaries, mainly in the stroma. For PCOS rats, the expression of DPP4 in the ovarian stroma was obviously stronger than that of the control group, and the over expression of DPP4 was estimated to have a certain correlation with the occurrence of PCOS. Additionally, in the rat ovarian tissue, we found that molecules associated with the TGF-β1/Smad2/3signal transduction pathway, which

is closely related to tissue fibrosis, showed notably stronger expression, and we observed stronger expression of the CTGF protein, which lies downstream. These molecules were intensely expressed in the fibroblasts and fiber cells of the ovarian stroma while weakly positive or negative in theca cells and granular cells. In a further analysis of mRNA and total protein levels, we found that the mRNA and total protein expression levels of DPP4, TGF-β1 and CTGF in the ovaries of PCOS rats were obviously higher than those in the ovaries of the control group, which showed that there were synergistic actions among DPP4, TGF-β1 and CTGF during the occurrence of PCOS.

In this study, we had also found that the expression intensities of DPP4, TGF-β1, Smad2, Smad3 and CTGF in the rat ovarian stroma were weakened in the PCOS treatment group after rats were treated with the DPP4 inhibitor Sitagliptin. The mRNA and total protein levels of DPP4, TGF-β1 and CTGF were noticeably decreased, but no difference was observed in the levels of Smad2 or Smad3, which was probably related to the regional distribution of Smad2 and Smad3, the small specimen quantity of this research, or the improper dosage of Siglitatin. This conclusion indicated that the DPP4 inhibitor Siglitatin could mitigate ovarian fibrosis in PCOS rats. This action might come into effect through the TGF-β1/Smad2/3 signal transduction pathway and its downstream functional element CTGF. Kosuke Kaji proposed that low-dose Siglitatin could inhibit the synthesis of collagen fibers through the ERK1/2, p38 and TGF-β1/Smad2/3 pathways during hepatic fibrosis inhibition. Siglitatin was also reported to alter different genes that are involved in inflammation, fibrosis and cell growth through the JAK/STAT signal transduction pathway[25,26]; thus, there are likely other pathways for DPP4 inhibitor-mediated anti-ovarian fibrosis.

Previous studies have shown that DPP4 can stimulate an increase in stromal cell-derived factor 1 (SDF-1, also called CXCL12).[27] SDF-1 increases cAMP levels, ROS and TGF-β1 levels within a tissue, thus activating the TGF-β1/Smad2/3 signaling pathway and its downstream elements, causing tissue fibrosis. On this basis, we infer that the antifibrosis mechanism of the DPP4 inhibitor Siglitatin may be that after inhibiting DPP4 expression, Siglitatin reduces SDF-1 levels and blocks the TGF-β1-Smad2/3 signaling pathway and its downstream element CTGF, thus alleviating tissue fibrosis. DPP4 is somewhat related to the occurrence and development of PCOS. PCOS patients usually suffer abnormal metabolism. DPP4 inhibitors can help patients with diabetes decrease IR, reduce glycosylated hemoglobin and reduce abnormally increased alanine aminotransferase and aspartate aminotransferase in the liver.[28] The inhibition of DPP4 expression is expected to reduce ovarian fibrosis and reduce islet resistance. For patients with PCOS combined with islet resistance, DPP4 inhibition can hopefully be a new option for treatment.

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Disclosure

No conflict of interest is declared.

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