Pathological characteristics of uterus in rats with polycystic ovary

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Background and Objective: Uterus of rat with polycystic ovary (PCO) may show pathological features. We provided pathological evidence for the rat uterus with NO-induced PCO.

Materials and Methods: Wistar rats (weighing 200-250 g) were kept diestrous to receive L-arginine (50 mg/kg) intraperitoneally (i.p.) for 9 days/once a day. Control group solely received saline (1 ml/kg, 9 days/once per day). At the end of the treatment period, all animals were surgically studied. The rats' ovaries and uteri were examined biometrically and collected in 10% formalin. The pathological data were collectively determined.

Results: The treated ovaries of rats showed polycystic characteristics when compared with the control. The uteri of treated rats also showed pathological changes as compared to those that belonged to the controls.

Conclusion: The pathological aspect of rat uterus may be linked with the cystic characteristic of ovary in PCO model. This study provides pathological evidence for uterus of rat with PCO.

1. Introduction

One of the endocrine disorders of women is known as polycystic ovary (PCO) which is characterized by polycystic feature of ovary along with the hyperandrogenism and ovulatory dysfunction (1,2). Also, one who suffers from PCO may show a higher exhibition of pro-inflammatory agents such as nitric oxide (NO) (3).

We have already shown that the chronic use of L-arginine, a precursor of NO, in rats with diestrous phase may induce the PCO alongside with lipid metabolism malfunction (3), the characteristics that mark the PCO syndrome (PCOS).

Since, by reviewing of literature, the uteri of those suffering from PCO have not been much studied, we sought to evidence pathologically the PCO model's uterus. In this work, the female Wistar rats were treated chronically with Larginine to provide the PCO model. Also, the experimental animals' uteri were examined to provide valuable data representing the involvement of NO in pathophysiology of the disorder.

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2. Materials and Methods

2.1. Animals

Wistar rats (body weight 200-250 g) were purchased from Pasteur Institute of Iran and were retained under standard conditions (21 ± 3°C and 12-h light/dark cycle) with food and water ad libitum. All experiments were approved by local Ethical committee.

2.2. Drugs

L-arginine (Merck Co., Germany) was injected i.p. for a 9-day period/ once per day. The vehicle (saline at 1 ml/kg, i.p.) was used in control group.

2.3. Female Cycle Test

Since female rats with 4-5 day sexual cycle are always in diestrous (4,5) unless in case of having mating with the male rat, so, the rats were kept virgin in the present study to avoid the change in female sexual cycle.

2.4. Drug administrations

Animals were randomly divided into the L-arginine (50 mg/kg), and saline control (1 ml/kg) groups (n=6). They were injected the agent or saline intraperitoneally (i.p.) once a day during the 9 days period.

2.5. Surgery procedure

The treatment groups were anesthetized by an overdose of diethyl ether. Then, a midline incision in the lower abdomen area was performed. The ovaries and uteri were biometrically examined and dissected out. They were collected in 10% formalin for histological examination.

2.6. Histological investigation

The collected tissues were processed and sectioned at a thickness of 3-4 µm. They were stained by Hematoxylin and Eosin (H&E) method (6). The thin sections were then dehydrated, cleared, and eventually mounted with entellane (Merck Co., Germany) and coverslipped. The prepared slides were evaluated with light microscope (Olympus, Japan) at 4-40X.

2.7. Image analysis

The photomicrographs were assessed in areas of 100-µm^2 with an aid of Image Tool program (UTHSCSA, version 2.03), the free image processing and analysis program for Microsoft Windows.

2.8. Statistical analysis

All data were first assessed by Kolmogorov-Smirnov (K-S) to show the equality to analysis by variance (ANOVA). The ANOVA was then performed using SPSS software (version 13.0; SPSS, Inc., Chicago, IL), followed by post-hoc test. Statistical significance was considered at $p< 0.05$. All data are expressed as Means ± SEM. The photos were examined in an area of 100-µm^2 using the Image Tool program.

3. Results

3.1. Histology

The ovaries from the L-arginine-treated group (50 mg/kg, chronically) showed cystic formations (Fig. 1B) as compared to control samples (Fig. 1A), the aspects certifying the polycystic ovary (PCO) example.

The uteri samples of those received chronically L-arginine (50 mg/kg) (Fig. 1C) presented pathological evidence to those belonged to the controls (Fig. 1D). Due to activation of the L-arginine-related metabolic pathway, the uterus wall revealed the aspects of swelling, the proliferation and angiogenesis, suggesting an inflammatory process involvement.

3.2. Biometrical value (diameters of uteri)

The uteri diameters were calculated in all groups. They showed changes in L-arginine-treated group as compared to those obtained from saline group. The uteri of rats treated with L-arginine showed a significant increase when compared with the control group ($p<0.05$) (Fig.2).

4. Discussion

This research study showed that chronic treatment of rats with a nitric oxide (NO) agent, L-arginine, induces the polycystic ovary (PCO) formation as well as the uteri inflammation.

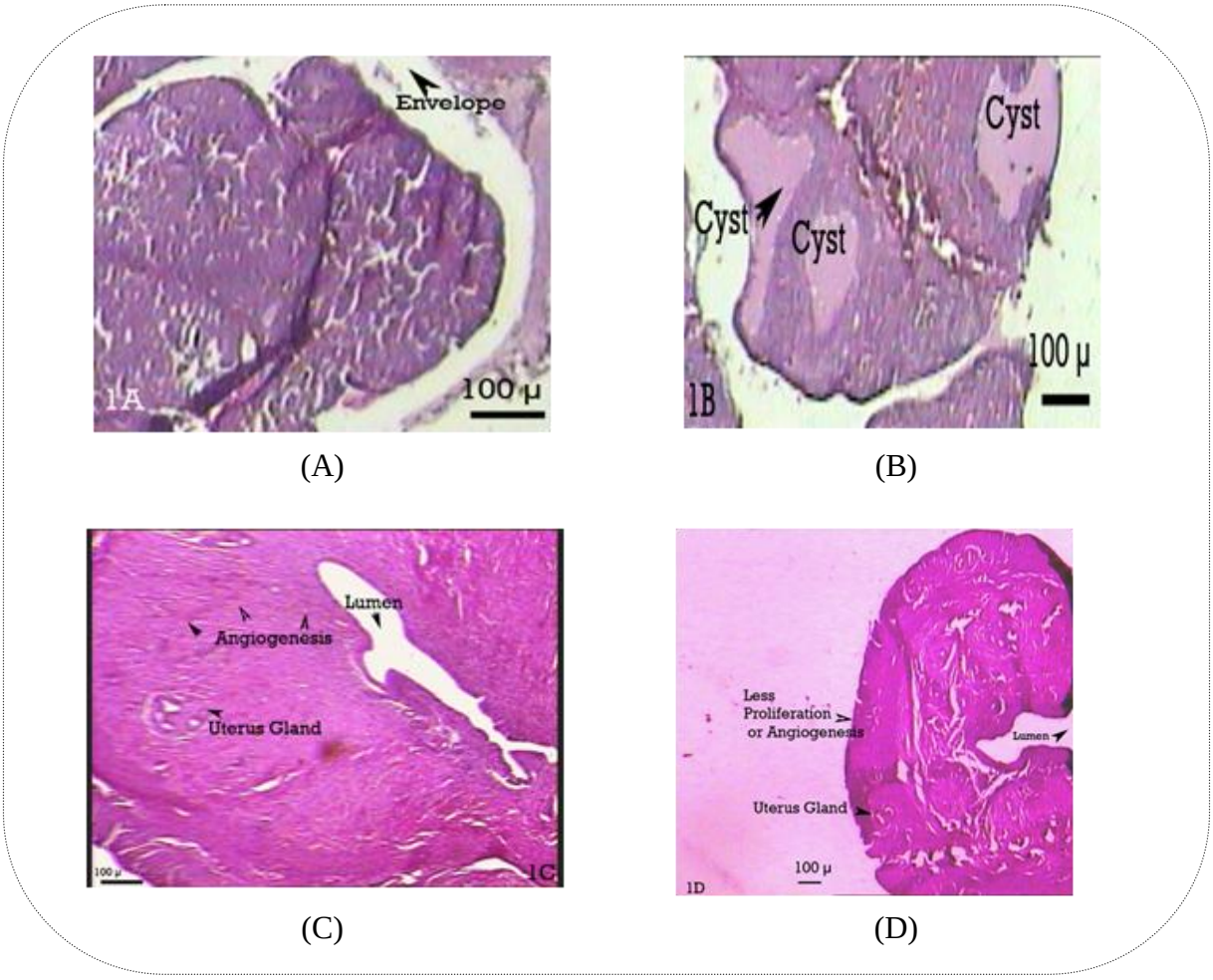

Figure 1. Photomicrographs of ovaries and uteri from control (A, C), and L-arginine-treated (B, D) rats.

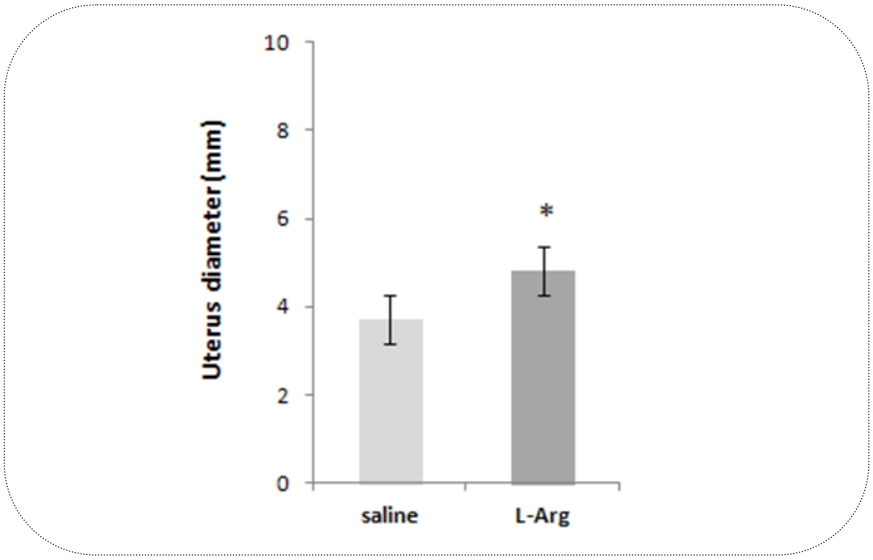

Figure 2. The diameter of uteri in rats. X axis denotes the control and experimental groups (n=6). Control was injected saline (1 ml/kg, i.p., 9 days/once per day). The experimental rats received L-arginine (50 mg/kg, i.p. for 9 days). Values are means ± S.E.M.* $p < 0.05$ vs. control (based on *Post-hoc* test).

The pro-inflammatory NO participates in endocrine physiological and pathophysiological events (7). The enzyme nitric oxide synthase (NOS) produces the NO by the oxidation of terminal guanidino nitrogen of arginine (7). We have already demonstrated the NOS activation with NADPH-diaphorase (8). The molecule NO is well introduced as a local inflammatory generator (9). The present work also provides support for a functional role of the NO in the ovarian and uterine events. This plan further supports our previous study (8), denoting that hyperactivity of enzyme NOS due to chronic usage of L-arginine induces polycystic formation in treated rats' ovaries. In agreement with this idea, it has been indicated that the presence of large cysts due to treatment by NO producer, L-arginine, accords with common characteristics of PCOS (10).

In addition of significant changes in feature of ovary, the uterus of the L-arginine-treated rats also showed differences as compared to the saline control group. These finding may suggest that the NO as a pro-inflammatory element may induce the inflammatory changes in uteri as well as ovaries. Although the exact effect of the L-arginine in this study remained elusive, the metabolic pathway may involve the NO which is known as a short-lived cytotoxic mediator (11). By viewing of the uterus diameter that increased in L-arginine-treated rats, it appears that inflammatory processes play crucial role in reproduction at all levels from the follicles and ovarian function to the accessory sex organs (i.e. uterus). We aimed to involve the NO by chronic use of L-arginine in PCO and pathology of rats' uteri. We have now evidenced that production of pro-inflammatory NO may induce significant change in the ovary and uterus parameters. It should be notified that role of NO in activation of NOergic neurons of the pelvic plexus has been previously shown (12). The NO has also been involved in the control of uterine smooth muscle via NOergic terminals (13). Based on our results, however, the exact mechanisms to involve the inflammatory processes in ovary and uterus events rest elusive.

In conclusion, this study indicates the pathological evidence in reproductive system of Wistar rats with PCO. Because of activation of the L-arginine-related metabolic pathway, the uterus wall illustrated the aspects of swelling, the proliferation and angiogenesis, suggesting an inflammatory process involvement.

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Conflict of interest

The authors state that they do not have any conflict of interest.

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