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Expression of matrix metalloproteinases and ovarian morphological changes in androgenized cyclic female guinea pigs

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ABSTRACT

This study was conducted to investigate expression of matrix metalloproteinases (MMPs) and ovarian morphological changes in androgenized cyclic female guinea pigs. Adult cyclic female guinea pigs were injected daily for 28 days with medium doses of testosterone propionate (TP; 1 mg/100 g), high doses of TP (2 mg/100 g), or saline (control). Serum concentrations of testosterone, estradiol (E_2), and progesterone (P_4) were measured. Histologic sections of ovaries were stained with hematoxylin–eosin and by immunohistochemistry. Expressions of steroidogenic acute regulatory protein, proliferating cell nuclear antigen, and MMP-2 and MMP-9 in the ovary were characterized by immunohistochemistry. After 28 days of TP injection, serum testosterone concentrations were increased dose-dependently. An appropriate dosage of TP could induce permanent anovulation in guinea pigs, making them a potential model for human polycystic ovary syndrome. MMP-2 and MMP-9 are jointly involved in the growth and atresia of ovarian follicles in cyclic guinea pigs. Increased numbers of atretic antral follicles in the ovary might be associated with the observed high expression of MMP-2 in androgenized cyclic guinea pigs.

1. Introduction

Polycystic ovary syndrome (PCOS) is the most common female endocrine disorder, affecting 5–10% of women of childbearing age (Norman et al., 2004). However, the pathogenesis of PCOS is unclear, and environmental factors might be involved. Several methods have been applied to produce rodent models of PCOS, such as treatment with androgens, estrogens, aromatase inhibitors, antiprogestogens (e.g., mifepristone), continuous illumination, and genetic modification (Shi and Vine, 2012). Estradiol valerate is commonly used to facilitate rat models of PCOS, because it produces an irregular estrous cycle, an atypical metabolic disorder, and polycystic ovaries (Quandt and Hutz, 1993; Stener-Victorin et al., 2000; Stener-Victorin and Lindholm, 2004; Manni et al., 2005). Continuous treatment with dihydrotestosterone can be used to one way to induce PCOS in obese rats, and can also cause typical hormonal disorders and ovarian morphological changes (Manneras et al., 2007). Thus, dihydrotestosterone treatment can be used to mimic PCOS with both ovarian and metabolic characteristics. Moreover, the insulin sensitivity and estrus cycle of model animals can be improved through wheel running exercise in rats (Benrick et al., 2013). A PCOS model induced by injection of testosterone did not cause ovarian shape changes or hormonal disorders simultaneously in proestrous female rats. Experiments have shown that the injection of testosterone into proestrous female rats inhibited ovulation. Injections with testosterone propionate (TP) for 28 continuous days led to high serum insulin levels and a significant reduction in the glucose/insulin ratio. The ovaries of such TP PCOS animals can contain many primordial follicles, while the preantral and antral follicles are well preserved. Thus, TP-PCOS animals can be used as models for investigating self-recovery from PCOS (Beloosesky et al., 2004).

Several experimental animals' models have been used in the simulation of follicle proliferation and metabolic symptoms of human PCOS through treatment with steroid hormones. These include non-human primates, rats, mice, and sheep (Abbott et al., 2012; Padmanabhan et al., 2010; Padmanabhan and Veiga-Lopez, 2011). Compared with rats and mice, guinea pigs have an estrus cycle more similar to humans. Moreover, ovarian and follicular differentiation occurs before birth in guinea pigs, but after birth in rats and mice (Padmanabhan et al., 2010). The secretion of ovarian hormones in rats and mice is not fully similar to humans. For instance, the synthesis of progesterone (P_4) in the human ovary can inhibit the development of a luteinizing hormone surge, but ovarian P_4 in rats and mice is a promoter of the luteinizing hormone surge

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(Kasa-Vubu et al., 1992; Levine, 1997; Gemzell-Danielsson and Marions, 2004). Thus, guinea pigs have great potential for simulating human PCOS.

Matrix metalloproteinases (MMPs) play important roles in follicular development and corpus luteum formation in the mammalian ovary. MMP-2 is involved throughout human oogenesis from primordial follicles, to mature follicles, through to corpus luteum formation (Vos et al., 2014). Moreover, active MMP-2 gene expression is required in the remodeling of ovarian tissues, during ovulation and in the formation of the corpus luteum (Deady et al., 2015). MMP-9 expression levels increase during follicle maturation, peaking after the first growth phase and remaining high after ovulation. The synthesis and secretion of MMP-9 mainly occur in granulosa cells at different stages of ovarian development. However, ovarian MMP-9 expression is regulated by growth factors, rather than hormones (Zhu et al., 2014). The overexpression of ovarian MMP-2 and MMP-9 and the down-regulation of tissue inhibitor of metalloproteinase (TIMP) might induce the proliferation of ovarian tumor cells (Sakata et al., 2000). Increased expression of ovarian MMP-9 can also induce metastasis of ovarian cancer cells. Therefore, in this study, injection of TP into guinea pigs was used as a potential model of human PCOS and the ovarian expression of MMP-2 and MMP-9 was studied.

2. Materials and methods

2.1. Animals and experimental design

Adult female albino guinea pigs (*Cavia porcellus)* were purchased from Jinhua Laboratory Animal Center (Jinhua, China). Eighteen guinea pigs with normal estrus cycles (16–18 days) were divided into three groups and injected daily with a medium dose of TP (1 mg/100 g; T-1 group), high dose of TP (2 mg/100 g; T-2 group) or normal saline (control group) for 28 continuous days. Then, the animals were euthanized by an anesthetic overdose with isoflurane (Foranew, Abbott Japan Co., Ltd., Tokyo, Japan) and blood samples were collected by cardiac puncture. The ovaries were removed and fixed in 4% paraformaldehyde for 36 h, and immersed in 70% alcohol. Histological sections (see Section 2.3) were stained with hematoxylin and eosin (HE) and immunohistochemistry (IHC). Blood samples were centrifuged at 5000 × *g* for 10 min and sera were stored at −70 °C until quantification of testosterone, estradiol (E_2), and P_4 by Adicon Clinical Laboratories Inc. (Hangzhou, China). All institutional and national guidelines for the care and use of laboratory animals were followed by the Institutional Animal Care and Use Committee of Nanjing Agricultural University.

2.2. Reagents

TP solution (Veterinary Medicine No. 110201054; 2003) was purchased from the Hangzhou Animal Medicine Factory (Hangzhou, China). MMP-2 (MAB-0244), MMP-9 (MAB-0245), and mouse IgG-horseradish peroxidase IHC kits (KIT-5910) were purchased from Fuzhou Maixin Biotech. Co., Ltd. (Fuzhou, China). Proliferating cell nuclear antigen (PCNA, SC-7907, lot: L5346) and steroidogenic acute regulatory protein (StAR, SC-25806, lot: I2308) were purchased from Santa Cruz Biotechnology Inc. (Dallas, TX, USA). Rabbit IgG IHC kits (SA2002, lot: 10C09A) and a streptavidin–biotin complex kit (SA2002, lot: 10C09A) were purchased from Wuhan Boster Bioengineering Co., Ltd. (Wuhan, China). All other reagents were analytically pure and made in China.

2.3. Histology and immunohistochemistry

Following fixation, ovarian samples were embedded in paraffin wax, and 5 μm serial sections were prepared and stained with H–E for histology. The numbers of primordial and antral follicles were counted on five sections for each sample.

To detect immunolocalized MMPs and steroidogenesis in ovaries, we performed IHC staining using monoclonal antibodies against MMP-2, MMP-9, StAR, and PCNA with streptavidin–biotin complex kits. Antibodies were diluted to 1:200 in phosphate-buffered saline containing 1% bovine serum albumin. Slides were immersed in 10 mM sodium citrate buffer (pH 6.0) and heated at 100 °C for 8 min in a microwave oven to perform heat-induced

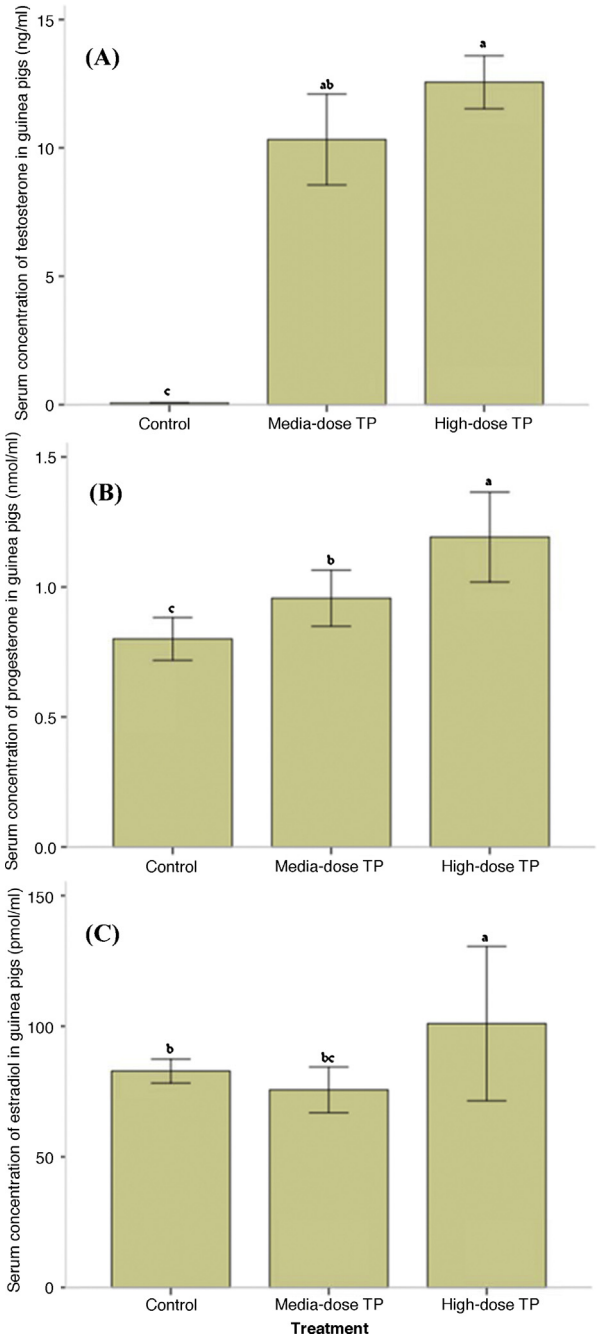

Fig. 1. Serum concentrations of testosterone, E_2 and P_4 in guinea pigs after 28 days of daily TP treatment. The control group: was injected subcutaneously once daily with saline; the T-1 group (medium-dose TP) received 1 mg/100 g body weight TP; the T-2 group (high-dose TP) received 2 mg/100 g body weight TP (the same below). Number of animals in each group, $n = 6$. Each value represents with the mean ± SEM. One-way analysis of variance (ANOVA) was used for analysis. Labels with differing superscripts of a, b and c represent significant differences between categories ($P < 0.05$) by Tukey test, while the same letters denote a lack of significance ($P > 0.05$).