Effects of Genistein on Gonadotropin and Insulin-like Growth Factor Expression in Female Rats (Postprint)

Authors: Zhen Jinglong, Chu Xiaoli, Zhang Tao, Cong Sha, Chi Xiaoxing

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Abstract

This study aimed to investigate the effects of genistein (GEN) on the expression of gonadotropins and insulin-like growth factors in female rats. Forty Sprague-Dawley (SD) female rats [body weight (200$±$20) g] were randomly divided into 5 groups (n=8): negative control (NC), GEN low-dose (L), medium-dose (M), high-dose (H), and positive control (PC) groups. The NC group received intragastric administration of peanut oil (used as the solvent for other groups); the L, M, and H groups received GEN at doses of 15, 30, and 60 mg/(kg BW·d), respectively, and the PC group received diethylstilbestrol at 0.5 mg/(kg BW·d). The experimental period lasted 30 days. Serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), insulin-like growth factor-1 (IGF-1), and insulin-like growth factor binding protein-1 (IGFBP-1) were measured by enzyme-linked immunosorbent assay (ELISA), while ovarian IGF-1 and IGFBP-1 mRNA expression levels were detected by real-time quantitative PCR. The results showed that, compared with the NC group, serum FSH and LH levels in the treatment groups exhibited an increasing trend, but the differences were not statistically significant ($P>0.05$), with effects consistent with the PC group. Serum IGF-1 content in the treatment groups was slightly decreased, but the difference was not significant ($P>0.05$), whereas the PC group showed a significant decrease ($P<0.05$). Serum IGFBP-1 content in the treatment groups was significantly or extremely significantly increased ($P<0.05$ or $P<0.01$), and the PC group was significantly increased ($P<0.05$). IGF-1 and IGFBP-1 mRNA expression levels in ovarian tissue were elevated in all treatment groups, with significant increases observed in the M and H groups ($P<0.05$), consistent with the changes in the PC group. These results indicate that GEN can increase serum FSH and LH levels, decrease serum IGF-1 levels, and increase serum IGFBP-1 levels in female rats, while simultaneously increasing IGFBP-1 and IGF-1 mRNA expression levels in the ovary. These indicators act synergistically on the ovary to promote follicular maturation and regulate ovarian function.

Full Text

Effect of Genistein on Expressions of Gonadotropins and Insulin-like Growth Factors in Female Rats

ZHEN Jinglong[1], CHU Xiaoli[1], ZHANG Tao[2], CONG Sha[1], CHI Xiaoxing[1]*

[1]College of Food Science, Heilongjiang Bayi Agricultural University, Daqing 163319, China
[2]Daqing Campus of Harbin Medical University, Daqing 163000, China

Abstract: This study investigated the effects of genistein (GEN) on gonadotropin and insulin-like growth factor expression in female rats. Forty female Sprague-Dawley rats weighing (200$±$20) g were randomly divided into five groups (n=8 per group): negative control (NC), GEN low-dose (L), GEN medium-dose (M), GEN high-dose (H), and positive control (PC). The NC group received peanut oil via gavage (used as solvent for other groups), while L, M, and H groups received GEN at 15, 30, and 60 mg/(kg BW · d), respectively, and the PC group received diethylstilbestrol at 0.5 mg/(kg BW · d). The 30-day trial assessed serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), insulin-like growth factor-1 (IGF-1), and insulin-like growth factor binding protein-1 (IGFBP-1) by enzyme-linked immunosorbent assay (ELISA), and ovarian mRNA expression of IGF-1 and IGFBP-1 by real-time quantitative PCR. Compared with the NC group, serum FSH and LH levels in treatment groups showed an upward trend without significant differences ($P>0.05$), consistent with the PC group. Serum IGF-1 decreased slightly in treatment groups ($P>0.05$) but significantly in the PC group ($P<0.05$). Serum IGFBP-1 increased significantly or extremely significantly in treatment groups ($P<0.05$ or $P<0.01$) and significantly in the PC group ($P<0.05$). Ovarian IGF-1 and IGFBP-1 mRNA expression levels increased in treatment groups, with significant elevations in M and H groups ($P<0.05$), mirroring the PC group pattern. These findings demonstrate that GEN can increase serum FSH and LH, decrease serum IGF-1, increase serum IGFBP-1, and upregulate ovarian IGFBP-1 and IGF-1 mRNA expression, collectively promoting follicular maturation and regulating ovarian function.

Keywords: genistein; follicle-stimulating hormone; luteinizing hormone; insulin-like growth factor-1; insulin-like growth factor binding protein-1; female rat

Introduction

Insulin-like growth factors (IGFs) primarily amplify gonadotropin effects on the ovary and play a crucial role in sustaining follicular growth during the mid-follicular phase when follicle-stimulating hormone (FSH) levels are low. IGFs stimulate ovarian cell mitosis, steroidogenesis, and inhibit apoptosis. The IGF family components IGF-1 and insulin-like growth factor binding protein-

1 (IGFBP-1) in blood are mainly synthesized and secreted by the liver, with expression regulated by various nutritional conditions and closely related to physiological status. IGFBP-1 primarily inhibits IGF-1 activity, while IGFBP-1 through IGFBP-6 can extend IGF-1 half-life in circulation. IGFBP-1 regulation of IGF-1 plays important roles in wound healing, and the IGF family is also implicated in diabetes, hyperthyroidism, immune system function, and cancer.

Plant estrogens include three main classes: isoflavones, lignans, and coumestans, found in legumes, plants, and seeds, with isoflavones being most abundant. Genistein (GEN), a highly active soy isoflavone component, exhibits structural similarity to endogenous estrogen and can bind estrogen receptors to produce estrogen-like effects.

Domestic research has extensively reported on soy isoflavone effects on animal ovaries. Dietary supplementation with 50 mg/kg soy isoflavones significantly improved laying rates in aging hens without adverse effects on egg quality or reproductive organs. Studies have shown that dietary daidzein at 50, 100, and 200 mg/kg improved fertilization and hatching rates in Gaohuang chickens without side effects. Other research demonstrated that soy isoflavones may improve ovarian function by increasing serum estradiol (E2) and β-endorphin levels. However, studies specifically examining GEN effects on female animal ovaries remain limited. One investigation showed that GEN and equol supplementation increased oocyte diameter and serum E2 levels in female beluga sturgeon, suggesting benefits for aquaculture development. Current research has focused primarily on GEN' s role in treating ovariectomized osteoporosis, menopausal hormone replacement therapy, and polycystic ovary syndrome, with few studies investigating its effects on ovarian functional genes and fertility in young female rats.

Our previous research demonstrated that GEN' s estrogen-like effects can regulate ovarian function in young, adult, and perimenopausal female rats. Animal experiments confirmed that GEN at 15, 30, and 60 mg/kg doses showed no significant effects on mouse ovaries or other vital organs. Building upon these findings, this study further investigated GEN' s regulatory effects on ovarian function in female rats by examining its impact on gonadotropins and IGFs, providing theoretical basis for understanding GEN' s role in ovarian function modulation and reproductive capacity.

Materials and Methods

1.1 Experimental Reagents, Basal Diet, and Main Instruments

GEN (99.82% purity) was purchased from Shanghai Ronghe Pharmaceutical Technology Development Co., Ltd. Diethylstilbestrol (DES, 99.4% purity) was obtained from Xi' an Tianzheng Pharmaceutical Excipients Co., Ltd., and peanut oil from Shandong Luhua Group Co., Ltd. To eliminate potential interference from dietary GEN and highlight treatment effects, a basal diet was used (composition shown in Table 1). TRIzol Reagent, SYBR Green PCR kit, and reverse transcription PCR kit were from Thermo Fisher Scientific,

and ELISA kits from Shanghai Yanjin Biotechnology Co., Ltd. Instruments included: TG-16M low-speed refrigerated centrifuge (Shanghai Luxiangyi Centrifuge Instrument Co., Ltd.), K-30 vortex mixer (Qingpu Luxi Instrument Factory), PRO-200 electric homogenizer (FLUKO), Nanodrop-2000 micro-spectrophotometer (Thermo), ABI-7300 real-time PCR system (ABI), and microplate reader (BioTec).

1.2 Experimental Design

Forty 49-day-old female SD rats weighing (200$\pm$20)$g(licensenumber:SCXK(Black)203—001)wererandomlydividedintofivegroups(n=8)basedonbodyweight:negativecontrol(NC),GENlow-dose(L),medium-dose(M),high-dose(H),andpositivecontrol(PC).Ratswerehousedindividually.TheNCgroupreceivedpeanutoilviagavage(us$
$d),respectively,andthePCgroupreceivedDESat0.5mg/(kgBW\cdot d).Animalsweremaintainedona14hlight:10hdarkcycleat(20\pm2)℃and(45\pm\$10)\%$ relative humidity with ad libitum access to water and feed for 30 days.

1.3 Sample Collection

After the experimental period, vaginal smears were examined to identify rats in diestrus. Selected diestrus rats were fasted for 12 h (water allowed), anesthetized with ether, and blood was collected from the abdominal aorta for serum separation. Ovarian tissues were harvested, snap-frozen in liquid nitrogen, and stored at -80 ℃.

1.4 Serum FSH, LH, IGF-1, and IGFBP-1 Measurement

Serum FSH, LH, IGF-1, and IGFBP-1 levels were determined by ELISA according to kit instructions.

1.5 Real-Time Quantitative PCR Analysis

1.5.1 Primer Design

Primers were designed using NCBI Primer Designing Tool and synthesized by Shanghai Sangon Biotechnology Co., Ltd. Primer information is listed in Table 2 .

1.5.2 Total RNA Extraction

Total RNA was extracted using TRIzol reagent according to manufacturer instructions. RNA purity and concentration were assessed by Nanodrop-2000 micro-spectrophotometer via OD value analysis.

1.5.3 cDNA Synthesis

The 25 μL reaction mixture contained: RNA-Primer Mix 12 μL, 5×RT Reaction Buffer 5 μL, 25 mmol/L dNTP 1 μL, 25 U/μL RNase Inhibitor 1 μL, 200 U/μL M-MLV Reverse Transcriptase 1 μL, Oligo(dt)18 1 μL, and ddH2O (DNase-free) 4 μL. Reaction conditions: 37 ℃ for 60 min, 85 ℃ for 5 min, and 4 ℃ for 5 min. cDNA products were stored at -20 ℃.

1.5.4 Real-Time Quantitative PCR

The 25 μL reaction system included: cDNA template 2 μL, SYBR Green Mix 12.5 μL, forward primer (0.1 mmol/μL) 0.5 μL, reverse primer (0.1 mmol/μL) 0.5 μL, and ddH2O 9.5 μL. Cycling parameters: 95 ℃ for 10 min; 40 cycles of 95 ℃ for 15 s and 60 ℃ for 45 s; followed by 95 ℃ for 15 s, 60 ℃ for 1 min, 95 ℃ for 15 s, and 60 ℃ for 15 s.

1.6 Statistical Analysis

Data were analyzed by t-test using SPSS 19.0 software and graphed with GraphPad Prism 5.0. $P<0.05$ was considered statistically significant and $P<0.01$ extremely significant.

Results

2.1 Effects of GEN on Serum FSH, LH, IGF-1, and IGFBP-1 Levels

As shown in Table 3 , compared with the NC group, serum FSH and LH levels in treatment groups exhibited upward trends without significant differences ($P>0.05$), consistent with the PC group pattern. Serum IGF-1 decreased in M and H groups but not significantly ($P>0.05$), while the PC group showed a significant reduction ($P<0.05$). Serum IGFBP-1 increased in all treatment groups, with significant elevation in L and H groups ($P<0.05$) and extremely significant increase in M group ($P<0.01$); the PC group also showed significant increase ($P<0.05$).

2.2 Effects of GEN on Ovarian IGF-1 and IGFBP-1 mRNA Expression

Figure 1 [Figure 1: see original paper] demonstrates single peaks in melt curves, indicating good primer specificity. Figure 2 [Figure 2: see original paper] shows that compared with the NC group, ovarian IGF-1 and IGFBP-1 mRNA expression increased in all treatment groups, with significant elevations in M and H groups ($P<0.05$). The PC group also exhibited significant increases in both mRNA levels ($P<0.05$).

[Figure 1: see original paper]

[Figure 2: see original paper]

Discussion

The regulation of IGF-1 activity by IGFBP-1 is complex. During fasting, low insulin inhibition combined with cortisol and glucagon stimulation of hepatic IGFBP-1 mRNA transcription results in high circulating IGFBP-1 levels. Since IGFBP-1' s affinity for IGF-1 exceeds IGF-1' s affinity for its receptor, elevated IGFBP-1 can inhibit IGF-1 binding to its receptor, thereby reducing IGF-1' s insulin-like metabolic activity. High fasting IGFBP-1 levels correlate with

lower prostate cancer risk, whereas high IGF-1 shows the opposite association. Notably, IGFBP-1 can directly interact with cell membrane receptor proteins independent of IGF-1 binding, stimulating Chinese hamster ovary cell migration. In teleost oocyte development and maturation, recombinant human IGF-1 induces germinal vesicle breakdown and promotes oocyte maturation in red seabream and spotted seatrout ovaries. In polycystic ovary syndrome, IGF-1 synergizes with insulin and LH to increase androgen production via paracrine, autocrine, and endocrine mechanisms, while decreased IGFBP-1 mRNA expression contributes to hyperandrogenemia and inhibits follicular maturation and estrogen synthesis. IGFBP-1 mRNA expression varies across tissues, being weaker in ovary than liver. IGF-1 mRNA expresses at low levels in theca cells of small antral and atretic follicles but not in dominant follicles, whereas IGFBP-1 mRNA appears only in granulosa cells of dominant follicles. Research indicates IGF-1 can act in autocrine or paracrine manners in cultured granulosa cells to enhance gonadotropin action in ovarian tissue. Gonadotropins are glycoprotein hormones regulating vertebrate gonadal development and sex steroid production, with LH and FSH from the anterior pituitary synergistically stimulating germ cell development and sex steroid synthesis in ovaries or testes.

In conclusion, genistein increases serum FSH and LH levels, decreases serum IGF-1, increases serum IGFBP-1, and upregulates ovarian IGFBP-1 and IGF-1 mRNA expression in female rats. These coordinated effects on the ovary promote follicular maturation and regulate ovarian function.

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Note: Figure translations are in progress. See original paper for figures.