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Research Highlight

Interference of Oral Contraceptive Pill on Male Reproductive System: Effects of 17α-Ethynylestradiol

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The structure feature of 17α-ethynylestradiol (EE2) exerts a higher binding affinity for estrogen receptor than natural estradiol (E2) and be widely used as an oral contraceptive pill. However, approximately 40% of the EE2 dose is excreted in the urine and feces. After exposure to the environment through wastewater, EE2 has been reported to interfere with reproductive function and other diseases in aquatic animals. Therefore, EE2 has been considered as one of endocrine-disrupting chemicals (EDCs). Long-term exposure to EDCs is accompanied by a number of diseases to raise a global challenge in health management, causing a tremendous financial burden. However, the effects of EE2 on male mammals is not well understood. In the present study, we found that rat reproductive system was inhibited after EE2 exposure. The results showed that EE2 inhibited testosterone production from rat Leydig cells through down-regulation of LH receptor- and calcium-mediated steroidogenic pathway. Moreover, the critical enzymes in cholesterol transportation were also abolished by EE2 exposure. Importantly, we found the organs of male reproductive system, including prostate and seminal vesicle, were atrophy and the sperm quality was reduced when rats were exposed to EE2. Taken together, our results in this study indicated a comprehensive understanding of how EE2 interfering male reproductive system in mammals.

Key words: 17α-ethynylestradiol, testosterone, male reproductive system

EE2 and EDCs

Seventeen-α-ethynylestradiol (EE2) is one of the commonly used medicines for female contraception. The pharmaceutical EE2 is a synthetic estrogen which exerts a higher estrogenic activity than estradiol (E2) (7, 14, 15). This structural feature lets EE2 pass into environment through body digestion and metabolism. It has been reported that approximately 40% of EE2 dose is excreted in the urine and feces (6). Exposure of EE2 to environment that has been reported to exert deleterious effects on the health of animals and human beings (1, 17). Therefore, environmental EE2 is reasonable to consider as one of endocrine-disrupting chemicals (EDCs). The risk of EDCs is a global issue. Abundant evidence has shown that EDCs interfere with endogenous endocrine processes through mimicking hormonal action, leading to cause reproduction-associated disorders and metabolic syndrome, as well as the rise of cancer incidence (2, 3, 12).

The effects of EDCs on the endocrine and reproductive systems in aquatic animals have been investigated widely. EE2 has also been reported to have detrimental effects on the reproductive system and confuse gender differentiation in fish (9, 11). Notably, long-term exposure to EE2 had been reported to interfere with the reproductive system and other biological functions in female rats (13, 16, 19). A previous animal study reported that abnormal development of the external genitalia was found in female offspring exposed to EE2 during the neonatal period. The offspring of female rats exhibited an irregular estrus cycle, including persistent estrus, which reduces reproductive capacity in pre-middle age (10). It has been mentioned that EDCs exposure has an impact on the male reproductive system (5). Although EE2 has been reported to alter of testosterone production in male fish (4), whether EE2 interferes with male reproductive systems is still unclear.

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Action Mechanism of EE2 on the Production of Testosterone

To assess whether EE2 affects the mechanism of testosterone production through alteration of steroidogenic enzyme activities in males, we designed an animal study to evaluate the effects of exposure to EE2 on the activity of steroidogenic pathway in rat Leydig cells (8). In our current study, we found that the levels of both basal and hCG-stimulated testosterone release were reduced in rat Leydig cells treated with EE2 in serial concentrations. The cAMP- and intracellular Ca^{2+}-mediated signaling in testosterone production were also abolished by EE2 treatment in rat Leydig cells. We further investigated how EE2 was involved in interfering with testosterone biosynthesis, two critical enzymes in steroidogenesis were detected. The results showed that EE2 reduced testosterone production might reduce the enzyme activity in cholesterol transportation mechanism, but it seems that affect the activity of 17β- hydroxysteroid dehydrogenase (HSD) was not affected.

In order to evaluate whether EE2 affects the circulatory concentration of testosterone, the male rats were administered with EE2 for 7 days. Compared to the control group, a dramatic decrease in the testosterone level of approximately 70% was observed after injection for 3 days with EE2 treatment. The sensitivity of hCG was monitored on day-4 post-EE2 treatment. The results showed that hCG-stimulated testosterone release was attenuated in all EE2-injected groups. Moreover, when hCG had been administered for 60 min in EE2-injected groups, the increased testosterone levels were reduced to the basal level of the control group. The opposite results showed that the plasma levels of LH were increased in all EE2-injected rats. We further isolated primary Leydig cells from rats treated with EE2 for 7 days and then evaluated the level of testosterone release. The results showed that the sensitivity of hCG in the primary Leydig cells exposed to EE2 for 7 days reduced and the expression of LH receptor was also reduced in rats exposed with EE2 for 7 days. Moreover, the LH receptor-regulatory downstream pathways, including adenylyl cyclase- and Ca2+-mediated pathways, were attenuated after EE2 exposure. The activity of steroidogenic enzymes was monitored in Leydig cells isolated from rats injected with EE2. After injection of EE2 in rats for 7 days, we found that EE2 exerts an inhibitory effect on LHR-induced adenylyl cyclase-cAMP regulation in rat Leydig cells. In addition, EE2 interfered with cholesterol transportation through down-regulating the expressions of steroidogenic acute regulatory protein (StAR) and cholesterol P450 side-chain cleavage enzyme (P450scc) and reducing P450scc enzyme activity.

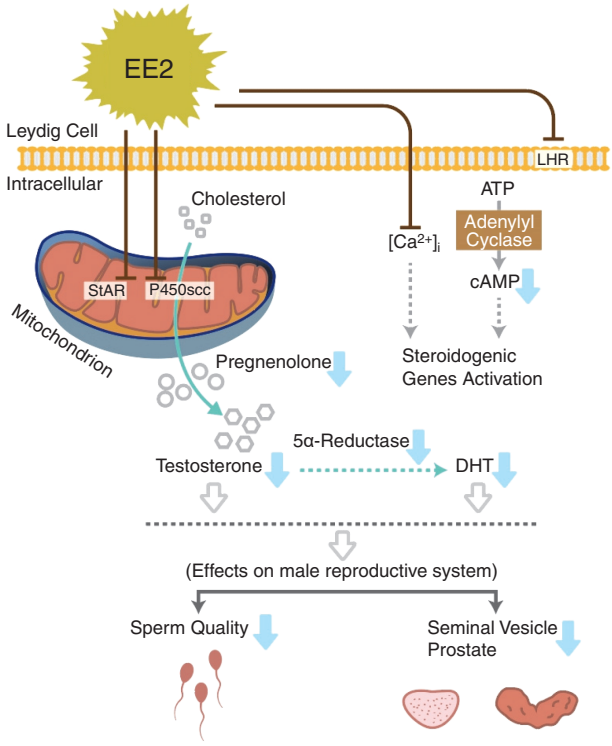

Fig. 1. Schematic summary of the mechanism underlying EE2-inhibited testosterone production in rat Leydig cells.

EE2 on Fertility and Activity of Male Accessory Organs

The lower circulatory DHT levels were parallel with the plasma testosterone levels after EE2 exposure. Previous reported showed that male fertility and the maintenance of spermatogenesis require androgen involvement (18). Based on the above results, the histopathological analysis of testes and the sperm quality in EE2-injected rats were further investigated. The feature of spermatogenesis and total sperm number in rat vas deferens were not altered, but we found that the sperm motilities in both vas deferens and epididymis were decreased after EE2 exposure. Moreover, we also found that EE2 exposure decreased the size of prostate and seminal vesicle. However, the weight of testes was no altered. The expression of type II 5α-reductase protein in both the prostate gland and the seminal vesicle was decreased by EE2 exposure.

Conclusion

Our study showed that EE2 exposure attenuated the male reproductive function through down-regulation

of androgen production. Mechanistically, EE2 interfered with LH receptor-mediated and calcium-activated steroidogenic pathways. EE2 also abolished the activities of steroidogenesis-associated enzymes, including P450scc and StAR protein. Importantly, the weight of prostate and seminal vesicle and sperm quality was reduced in rats exposed to EE2. Hence, we propose a schematic model of the mechanism underlying EE2-inhibited testosterone production in rat Leydig cells, as shown in Figure 1.

Conflicts of Interest

The authors declared that there is no conflict of interesting regarding the publication of this article.

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