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Review

Zearalenone impairs the male reproductive system functions via inducing structural and functional alterations of sertoli cells

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ABSTRACT

The aim of this study was to investigate the effects of ZEA on the cytoskeletal structure, and factors specifically expressed by Sertoli cells. Primary Sertoli cells from rats aged 18–21 days were exposed to increasing ZEA concentrations (0, 5, 10, 20 μg mL^{-1}) for 24 h. The results of immunofluorescence showed disruption of α-tubulin filaments and F-actin bundles, and damage to the nucleus of Sertoli cells on exposure to ZEA. In the control group, the protein level expression of androgen-binding protein (ABP), transferrin, vimentin, N-cadherin, and follicle-stimulating hormone receptor (FSHR) were decreased significantly ($p < 0.05$, $p < 0.01$). The mRNA levels of *ABP*, *transferrin*, *vimentin*, *N-cadherin*, and *FSHR* varied significantly in the experimental group ($p < 0.05$). The results of enzyme-linked immunosorbent assay indicated a significant decrease in the levels of inhibin-β and transferrin in the cultural supernatants ($p < 0.05$). Additionally, the ultrastructural analysis indicated the absence of mitochondria and Golgi apparatus, and presence of vacuoles in the cytoplasm. These findings showed that ZEA treatment can damage the cytoskeletal structure and affect specific secretory functions of Sertoli cells, which may be an underlying cause of ZEA-induced reproductive toxicity.

Contents

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1. Introduction

Zearalenone (ZEA) is a non-steroidal estrogenic mycotoxin produced by several fungi of the Fusarium sp. that contaminates cereal crops such as maize, barley, wheat, oats, sorghum, and sesame grains across the world (Tabuc et al., 2009). ZEA is genotoxic, immunotoxic, and tumorigenic, and it affects reproductive and developmental processes (Liang et al., 2015). It decreases fertility, reduces litter size, alters the weight of adrenal, thyroid, and pituitary glands in offspring, and changes the progesterone and estradiol levels (Tiemann and Danicke, 2007). Recent studies have shown that reproductive disorders in humans and farm animals with hyperestrogenic syndrome are attributed to ZEA (Minervini and Dell'Aquila, 2008).

Testis is a male reproductive organ that is targeted by many reproductive toxins. Sertoli cells, located in the seminiferous tubules of the testes, provide nutrition and morphogenetic support to the germ cells during spermatogenesis (Liu et al., 2014a). Both Sertoli cells and spermatogenic cells are critical for proliferation, differentiation, and survival of the germ cells (Cheng and Mruk, 2012). Morphometric analyses have shown that each Sertoli cell in adult rats provides structural and nutritional support to about 30–50 developing germ cells (Feng et al., 2010). Some secretions synthesized by Sertoli cells regulates or responds to pituitary hormone release, thereby influencing spermatogenesis further (Johnson et al., 2008). Another important function of Sertoli cells is to establish the blood-testis barrier (BTB), which provides an exclusive and stable environment for germ cell development. Thus, any agent that can impair the viability and/or function of Sertoli cells may profoundly affect spermatogenesis (Liu et al., 2014a).

ZEA can reduce the number of germ cells and Sertoli cells, alter the morphology of testis, cause testicular cells to differentiate abnormally, and affect fertility (Koraichi et al., 2013). Several studies have demonstrated that ZEA and its metabolites alter hormone production, including testosterone, thus acting as endocrine disruptors (Frizzell et al., 2011).

Studies in various animals have shown that ZEA and its metabolites exhibit estrogenic and anabolic activities. The strong estrogenic effects exhibited by ZEA are due to its competition with 17-β-estradiol for cytosolic estrogen receptors present in the uterus, mammary gland, hypothalamus, and pituitary gland (Ayed et al., 2013). Yang and colleagues proved that ZEA reduces testosterone secretion both in-vitro and in-vivo (Yang et al., 2007). ZEA activates the nuclear estrogen receptor signaling pathway to limit Nur77 expression, indirectly disturbs the transcription of steroidogenic enzymes (Liu et al., 2014b), activates the mitochondrial pathway of apoptosis by proteins from the Bcl-2 family and increases autophagy in Leydig cells. While low dose of ZEA can activate autophagy, high dose causes apoptosis in Leydig cells (Wang et al., 2014). Little is, however, known about the effects of exposure of ZEA on the cytoskeleton and function of Sertoli cells. This study investigated whether exposure of ZEA has adverse effects on cytoskeleton-associated molecules such as F-actin, α-tubulin, vimentin, and expression of specific proteins including N-cadherin, androgen binding protein (ABP), transferrin, follicle stimulating hormone receptor (FSHR), and inhibin-β.

2. Materials and methods

2.1. Reagents

ZEA, collagenase type I, bovine serum albumin (BSA) and oil red O were purchased from Sigma–Aldrich (St. Louis, MO, USA). Dulbecco's modified Eagles medium with Hams F-12 nutrient mixture (1:1 ratio; DMEM-F12) was obtained fromGIBCO BRL (Grand Island, NY, USA). The polyclonal antibodies against FSHR(sc-13935), vimentin(sc-6260), N-cadherin(sc-7939), transferrin(sc-30159), and ABP(sc-32891) were obtained from Santa Cruz Biotechnology Inc,(Santa Cruz,CA). All other chemicals and reagents were analytical grade and were obtained commercially.

2.2. Sertoli cell preparation

18–1 days male Wistar rats were purchased from Comparative Medicine Center of Yangzhou University (experimental animal production license number: SCXK2012 -0004). They were kept under controlled temperature (25 °C) and light (12 h light:12 h darkness) conditions. The animals were fed Xietong mouse feed (Xietong Co., Jiangsu, China) and water ad libitum until sacrifice. Surgical procedures were carried out under anesthesia with pentobarbital sodium (0.9%, 0.5 mL/100 g body weight; Solarbio, Beijing, China). The abdominal cavity was opened with sterile scissors and the testis was removed by cutting the spermatic cords. The excised testis was immersed in cold phosphate buffer solution for further steps.

2.3. Isolation and purification of primary Sertoli cells of rats

The testis of the rats was decapsulated with fine forceps, cut into pieces, and digested using 0.25% trypsin and 0.1% collagenase in DMEM-F12 at 37 °C for about 15 min in a thermostatic shaking water bath. After incubation, DMEM-F12 with fetal bovine serum (FBS) was added to each tube to stop the enzyme digestion. The resulting liquid was filtered using stainless steel trap valves (100 screen mesh) into sterile centrifuge tubes and centrifuged at 800 × *g* for 8 min. The sediment was washed thrice in DMEM-F12. The cells were resuspended in the culture medium (DMEM-F12 with 10% FBS, 100 IU/mL penicillin and 100 μg mL^{-1} streptomycin). After counting, the cells were plated in 6-well plates (2.5×10^6 cells/well) or 24-well plates (2×10^5 cells/well) and incubated at 37 °C in a humidified atmosphere of 5% CO_2. After 24 h, the cells were transferred serially into fresh culture medium. After 48 h of incubation, contaminating spermatogenic cells were lysed with a hypotonic