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Zearalenone and 17 β-estradiol induced damages in male rats reproduction potential; evidence for ERα and ERβ receptors expression and steroidogenesis

Elmira Adibnia [a], Mazdak Razi [a, *], Hassan Malekinejad [b, c]

[a] Department of Comparative Histology & Embryology, Faculty of Veterinary Medicine, P.O. Box: 1177, Urmia University, Urmia, Iran
[b] Department of Pharmacology & Toxicology, Faculty of Pharmacy, Urmia University of Medical Sciences, Urmia, Iran
[c] Department of Pharmacology & Toxicology, Faculty of Veterinary Medicine, P.O. Box: 1177, Urmia University, Urmia, Iran

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ABSTRACT

The estrogen receptors (ERs)-dependent effects of Zearalenone (ZEA) on structure and function of the testis as well as sperm parameters were compared with 17-β estradiol as endogenous substance. For this purpose, 30 mature male rats were assigned into five groups as; control (appropriate volume of normal saline, i. p.), ZEA-received (1, 2 and 4 mg/kg, b. w., i. p.) and 17 β-estradiol (E_2)-received (appropriate dose of 0.1 mg/kg, i. p.). Following 28 days, the mRNA levels of estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ) in the testis and sperms and the expression of them at protein levels in testicles were estimated. Mitochondrial content of germinal epithelium, Leydig cells steroid foci, sperm quality parameters and serum level of testosterone were assessed. Fluorescent techniques were used for analyzing apoptosis and mRNA damage in necrotic cells. ZEA reduced the mRNA and protein levels of ERα in testicles while up-regulated the ERβ expression. The mRNA level of ERα decreased in sperms of ZEA and E_2-received animals. No remarkable changes were found for ERβ expression in sperms from ZEA and E_2-received animals. ZEA reduced the Leydig cells steroidogenesis, mitochondrial content of germinal cells and elevated cellular apoptosis and necrosis dose-dependently. E_2 reduced the testosterone concentration, enhanced the apoptosis and reduced sperm quality. Our data suggest that ZEA-induced detrimental effects in the structure and function of testis, may attribute to changing the ERs expression at mRNA and translational level.

1. Introduction

Zearalenone (ZEA) is a mycotoxin produced by several species of *Fusarium* molds, such as *F. culmorum*, *F. crookwellense*, *F. equiseti and F. semitectum* that grow on oats, corn, and hay, when exposed to high humidity. Chronic exposure to ZEA has been reported to cause degenerative alterations in the reproductive tracts of both laboratory and domestic animals (Zinedine et al., 2007; Waskiewicz et al., 2012). Indeed, exposure to ZEA leads to testicular germ cell apoptosis (Lubahn et al., 1993; Yuan et al., 2010). Accordingly, single dose of ZEA (5 mg/kg b. w.) resulted in germ cells apoptosis and affected the sperm quality in dose-dependent manner (Yuan et al., 2010). Previous reports showed that chronic exposure to ZEA and other estrogenic compounds during fetal and neonatal development resulted in reduced sperm counts (Hess et al., 1997; Kim et al., 2003; Vrooman et al., 2015), cryptorchidism, epididymal defects, impaired fertility, and an increased incidence of testicular cancer (Yang et al., 2007a, 2007b; Takamura et al., 2007; Santos et al., 2013). In this line, Filipiak et al. (2009) performed an *in vivo* study and compared the ZEA, 17-β estradiol (E_2) and diethylstilbestrol (DES)-induced damages (Filipiak et al., 2009). Authors declared that E_2 and DES remarkably reduced the numbers of spermatogonia, spermatocytes and Sertoli cells. However, ZEA only reduced the numbers of spermatogonia and Sertoli cells.

In fact, the main impact of ZEA is related to its estrogen-like activity. ZEA and its metabolites, α-zearanenol (α-ZOL) and β-zearalenol (β-ZOL), compete with17 β-estradiol (E_2) for the specific binding sites of estrogen receptors (ERs). Several investigations have demonstrated that binding of ZEA and its metabolites initiates cascade of events known to follow estrogen stimulation (Korach,

* Corresponding author.
E-mail address: Mazdak.razi@gmail.com (M. Razi).

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2000; Takamura et al., 2007; Filipiak et al., 2009; Santos et al., 2013). Therefore, ZEA is known as a mycoestrogen that exerts estrogen like effects. In this regard exposure to estrogens, estrogen like substances, so called xenoestrogens is recently gained raising attentions for their detrimental impacts on male reproductive system.

Estrogen receptors (ERs) are the ligand-activated nuclear receptor family that are known to have affinity for the other steroid hormones, thyroid hormone, vitamin D, and retinoids (Pelletier et al., 2000; Agarwal et al., 2008; Markov and Laudet, 2011). Two forms of ERs have been reported in testicular tissue as ERα and ERβ. Previous reports showed that the ERα is expressed in Leydig cells and spermatocytes of adult rats and mice (Hall and McDonnell, 1999; Chimento et al., 2014; Takamura et al., 2007). However, ERβ is found in pachytene spermatocytes and round spermatids (Kuiper et al., 1998; Hall and McDonnell, 1999; Pettersson and Gustafsson, 2001). ERs in interaction with aromatase (cytochrome P450 enzymes family), are involved in estrogen-induced pathways for controlling spermatogenesis and spermiogenesis process (Kuiper et al., 1998; Hall and McDonnell, 1999; Chimento et al., 2014). There is considerable selectivity in ERs affinity to bind with different estrogenic ligands such as phytoestrogens and/or estrogen-like chemicals (Pettersson and Gustafsson, 2001; Pavao and Traish, 2001; Kabuto et al., 2004). Although the high tendency of ZEA to binding with ERs is known to be responsible for ZEA-induced detrimental effects, the impact of ZEA on expression levels of ERα and ERβ remained relatively unknown. In other words, by considering the effects of agonists and/or antagonists on ERs transcription, there are differences between ER subtypes (Grand et al., 2015). On the other hand, ERs (ERα and ERβ) recruit different coactivators and ligands in order to exhibit their functional properties (Zhao et al., 2005; Rastinejad et al., 2013).

The negative feedback of androgens on gonadotropins is mainly depends on their aromatization to E_2 (Hayes et al., 2000, 2001). Therefore, any increase in serum level of E_2 (resulting by administrating exogenous estrogen and/or estrogen-like agents) is able to suppress secretion of gonadotropins (Handelsman et al., 2000). Moreover, E_2 has been shown to inhibit the 17alpha-hydroxylase/C17, 20-lyase (P450 17, CYP 17) activities in Leydig cell that in turn is able to inhibit steroidogenesis required for testosterone biosynthesis (Brinkmann et al., 1980; Saunders et al., 1997). Hence, the present study was performed to evaluate the dose-dependent effect of ZEA on ERs expression at the testicular tissue and corresponding sperm samples. In order to illustrate the probable difference between ZEA and E_2, the ZEA-induced alterations in ERs expression compared with E_2-induced changes. Moreover, we aimed to uncover the effect of ZEA on Leydig cells steroidogenesis by comparing the histopathological and biochemical changes resulted from the E_2 administration. Ultimately, any changes due to the administration of both compounds on sperm quality parameters were also evaluated.

2. Materials and methods

2.1. Chemicals

ZEA (Cat No: Z 2125) was purchased from sigma chemical CO. (Sigma Aldrich, Germany). The acridine-orange was purchased from sigma chemical Co. (Sigma-Aldrich, USA). The FITC-conjugated1-anilinonaphthalene-8-sulphonate was obtained from Life technologies Co. (Thermo Fisher Scientific, Turkey). The rabbit anti-mouse primary antibodies for ERα and ERβ were purchased from Life Teb-Gen Co. (Biocare, USA). The DAB chromogen was from Agilent technologies Co. (DAKO, Turkey). Mounting medium for immunohistochemical analysis (VECTASHIELD) was from Vector Laboratories (Vector Laboratories, USA). Acid fuchsine and methyl-Blue were assigned from Pajohesh Asia Co, Iran. The TCM199 cell culture medium was purchased from Sigma chemical CO. (Sigma-Aldrich, Germany).

2.2. Animals and experimental design

Following one week acclimation, 30 mature male Wistar rats with average weight of 210–220 g were divided into five groups including (NO: 6 rats for each group); Control (0.3 ml, normal saline containing the same concentration of ZEA solvent, ip) and test groups. The animals in test group received different doses of ZEA, which was primarily dissolved in ethanol and the final concentration of solvent was not exceeded from 5% of the administered normal saline. The diet and water were given *ad libitum* and all stress factors were reduced into minimum and the animals were kept in standard condition (constant temperature and 12-h light). All animals were fed on soy-free diet (Mahabad-Konsantere, Iran). The test group subdivided into four groups as; low dose ZEA-received (1 mg/kg b. w., ip), medium dose ZEA-received (2 mg/kg b. w., ip), high dose ZEA-received (4 mg/kg b. w., ip) and E_2-rceived (0.1 mg/kg, b. w., ip) groups (Cheraghi et al., 2015). All animals were treated with chemicals for 28 continuous days. The experimental protocols were approved by the ethical committee of Urmia University number AECVU/135/2014.

2.3. Blood and tissue sampling and serum preparation

Following 21 days, blood samples were collected directly from heart by inducing a light anesthesia using diethyl ether. Then, the samples were centrifuged at 3000*g* for 10 min to obtain the serum. The serum samples were stored at −20 °C for further analyses. The animals then were euthanized by using CO_2 gas. The testicular tissues were dissected out under high magnification produced by Stereo Zoom Microscope (SZ×16-olympus, Japan) and ultimately dissected free from surrounding epidydimal tissue. One half of the tissues were fixed in Bouin's fixative for histological investigations and the other halves were stored at −70 °C for further analyses.

2.4. Histological analyses

Following tissue passage by using ascending alcohol concentrations (Ethanol, 70%, 80%, 90%, 96% and 100%) the testicular samples were paraffin embedded and cut (5–6 μm) by rotary microtome (Microm, GMBH, Germany). The sections were stained with Iron-Weigert (Pajohesh Asia., Iran) for detection of germinal epithelium's nuclei in the testis. The prepared slides were analyzed under light microscope by multiple magnifications (400 × and 1000 ×). The Leydig cells number per one mm^2 of interstitial connective tissue were numerated. The percentage of seminiferous tubules with more than 3–4 germinal layers and percentage of tubules with normal spermiogenesis were considered as positive tubular differentiation index (TDI) and positive spermiogenesis index (SPI), respectively. The percentage of tubules with positive repopulation index (RI), as the ratio of active spermatogonia (spermatogonia type B with light nuclei in Iron-Weigert staining technique) to inactive spermatogonia (spermatogonia type A with dark nuclei in Iron-Weigert staining) was calculated.

2.5. Histochemical analyses for Leydig cells steroidogenic activity

In order to uncover the intra-cytoplamic steroid foci in Leydig cells, the fluorescent staining for steroidogenic compounds was performed according to Life technologies., Lipid TOX. In brief; the frozen section prepared slides were hydrated. The sections were