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Prenatal exposure to zearalenone disrupts reproductive potential and development via hormone-related genes in male rats

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ABSTRACT

The present study investigated the reproductive and developmental toxicity of male offspring induced by prenatal ZEN exposure and explored the possible mechanism. 64 pregnant rats were allocated into four groups and fed with ZEN contaminated (0, 5, 10 and 20 mg/kg) diet during the whole gestation period. The results showed that, F1 male foetal viability was not affected while newborn bodyweight (BW) was significantly decreased after prenatal exposure to ZEN. Decreased BW was found on postnatal day (PND) 21 but not on PND 63 in ZEN exposed male rats. Moreover, adult testis weight increased with seminiferous tubules atrophy as well as decreased spermatocytes and mature sperms (35% and 31%) in ZEN-treated rats. Meanwhile, circulating levels of luteinizing hormone and testosterone decreased while estradiol increased in ZEN-treated rats. These impairments concurred with down-regulations of 3β-HSD and StAR in both mRNA and protein levels in weaned and adult testis. Furthermore, gene and protein expressions of GnRHr and Esr1 were inhibited in the ZEN-treated foetal brain. These results suggested that prenatal ZEN exposure disrupted the system regulating the reproductive hormones and testis development through hormone related genes, which may result in a reproductive dysfunction in adult male offspring.

1. Introduction

Over the past few decades, the increasing incidence of reproductive disorders observed in vertebrates has raised concern about the role of substances known as environmental endocrine-disrupting chemicals (EDCs). The EDCs are referred to naturally occurring substances (i.e., phytoestrogens and mycoestrogens) or synthetic chemicals that can interfere with the body's endocrine system and exert various reproductive effects (Diamanti-Kandarakis et al., 2009; Salian et al., 2011). Exposure to EDCs and in particular to xenoestrogens, leads to an increasing incidence of reproductive system disorders in animals and humans (Delbès et al., 2006). Zearalenone (ZEN), an environmental xenoestrogen, is a non-steroid estrogenic mycotoxin produced by species of Fusarium fungi (Bennett and Klich, 2003; Richard, 2007), and is one of the most prevalent mycotoxins that contaminate staple food, especially cereals in human and animal diet (Zinedine et al., 2007). High concentrations of ZEN (up to 600 mg/kg) in feed have been reported (Herrman and Walker, 1999; Kowalska et al., 2016; Zinedine et al., 2007). For humans, food ZEN contamination levels are usually in the range of μg/kg and low mg/kg, however, there are still serious ZEN contaminations in parts of Africa, Asia and North America, with the highest reported reaching 15 mg/kg (Sangare-Tigori et al., 2006; Zhao et al., 2013; Zinedine et al., 2007). Following oral administration, ZEN is rapidly absorbed from the gastrointestinal tract to the bloodstream and distributed to organs after passing through the liver. ZEN is mostly metabolized to α- and β-zearalenol (ZOL) in the liver by 3α and 3β-hydroxysteroid dehydrogenases (HSDs), respectively (Deng et al., 2012; Fink-Gremmels and Malekinejad, 2007). ZEN and its metabolites can competitively bind to estrogen receptor due to the structural similarity with 17β-estradiol (E_2), and activate the transcription of oestrogen-responsive genes in many organs, especially in the gonads (Bovee et al., 2004; Etienne and Dourmad, 1994; Metzler et al., 2010; Mirocha et al.,

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1981). α-ZOL has been proved to be the dominant ZEN derivative in humans (Adewale et al., 2009; Mirocha et al., 1981), rats, mice (Bravin et al., 2009) and pigs (Kuiper-Goodman et al., 1987). ZEN and its metabolites' oestrogenicity cause several functional and morphological changes in the reproductive organs and lead to numerous reproductive problems in both female and male animals (Belli et al., 2010; Benzoni et al., 2008; Yang et al., 2007b). As well, ZEN could disturb the prenatal development of mice and the estrous cycle, as well as increase the number of anovulatory rats (Arora et al., 1981; Nikaido et al., 2003).

Studies have reported that female is more susceptible to ZEN than male (Collins et al., 2006; Kuiper-Goodman et al., 1987). It has been well documented that female foetus exposure to ZEN causes follicle damage and premature oocyte depletion, therefore comprise a health risk for young offspring (Gao et al., 2017; Schoevers et al., 2012). However, the toxicity of ZEN on male should not be underrated, lower testicular weight and decreased motility of spermatozoa were found in boars and mice after continuous ingestion of low ZEN concentrations (Yang et al., 2007a; Young and King, 1986). Reduced fertility in the offspring may be the most obvious consequence of prenatal exposure to toxic environmental chemicals. It has been reported that male mice exposed prenatally to diethylstilbestrol had an excess prevalence of malformations of the genitalia and infertility (McLachlan, 1977; McLachlan et al., 1975). Likewise, ZEN and its metabolites can transfer into the foetus during gestation period by the placenta, mediate abnormalities in foetal growth and development, so that lead to impaired development, reduced litter size and foetal malformation (Kiessling and Pettersson, 1978; Young et al., 1990; Zhang et al., 2013). In human males, an excess rate of minor malformations of the genitalia has been associated with prenatal exposure to xenoestrogens (Gill, 1988). Furthermore, foetus growth was restricted in rats with detectable ZEN residual placentas, whereas normal with no ZEN residues (Zhang et al., 2013).

Despite the numerous studies about ZEN toxicity in male animals, most investigations were focused on the direct ZEN exposure toxicity, and little was known about the roles of prenatal ZEN exposure on the male reproduction. Therefore, the present study was conducted to evaluate whether prenatal exposure to ZEN was associated with reduced reproductive potential and disrupted development on male offspring. By observing testis structure, sexual hormones secretion and expressions of related genes and proteins, the detailed toxicity of prenatal ZEN were investigated in male rats.

2. Materials and methods

All procedures were in accordance with the National Research Council Guide (Clark et al., 1996), and approved by the Scientific Ethics Committee of Huazhong Agricultural University. The project identification code is HZAURA-2015-006.

2.1. Animals and treatments

ZEN was purchased from Sigma-Aldrich (St. Louis, MO, USA). Sprague-Dawley (SD) rats were obtained from Wuhan Centers for Disease Prevention & Control (Wuhan, China). The weights of the female rats ranged from 200 to 210 g. After a period of acclimating, they were mated overnight with males of the same strain.

Pregnant rats at gestational day (GD) 0 were individually housed under controlled and standardized conditions, with 12-h light-dark cycles (0700–1900 light). A total of 64 pregnant rats were divided into 4 groups (control, 5 ZEN, 10 ZEN, 20 ZEN), and received diets containing different concentrations of ZEN (0, 5, 10 and 20 mg/kg) through gestational days (GD 0–20). Water and feed were provided *ad libitum* during the experiment. Body weight (BW), average daily intake (ADI) and average daily gain (ADG) were recorded and calculated. The rationale for the dose selection: 5 mg/kg ZEN diet was about the dose of 0.5 mg/kg/day, it's the no-observed effect level (NOEL) of ZEN in rats (Collins et al., 2006); 10 and 20 mg/kg ZEN were 2 and 4 times of NOEL, which were also based on the levels reported in contaminated foods (Panel, 2011; Zinedine et al., 2007). The pregnant rats were fed on a regular diet containing no ZEN during the lactation phase of the study.

Eight pregnant rats from each group were sacrificed on GD 20 by cervical dislocation in order to collect placentas and foetuses (n = 12), while the other dams (eight of each group) were allowed to deliver and care for their pups. At birth, each pup was sexed, weighed, and identified. The litter size was balanced to 8 with half males and females. All pups were breastfed, weaned on a postnatal day (PND) 21. Only male rats were investigated in the study. Two weaning rats of each dams were sacrificed by cervical dislocation (n = 16). The remaining F1 male rats were left to be sexually mature and slaughtered (n = 16) at PND 63 (9 weeks).

Brains and testis of weaned and adult rats were sampled. Blood samples of adult F1 rats were immediately centrifuged following collection. All the samples were labeled and frozen at −80 °C until further analysis.

2.2. Testis histopathological analyses and germ cells quantitative analyses

The testis was sampled and fixed in 10% neutral-buffered formalin for 48 h. Then, the organs were processed for paraffin embedding, sectioned (thickness, 5 mm) and stained with hematoxylin and eosin (H &E) (Nikaido et al., 2004). The slides were observed under 100× or 200× magnification using an optical microscope (Nikon, Tokyo, Japan). A quantitative analysis of germ cells in each seminiferous tubule was carried out with Image J 1.49 (Clermont and Morgentaler, 1955; Li et al., 2017). The number of germ cells (including sertoli cell, spermatogonia, spermatocyte, spermatid and mature sperm) were counted in five fields per testis (n = 8) based on H&E staining, and the results were expressed as mean number of cells per seminiferous tubule.

2.3. Hormone levels detection

E_2 (estradiol), LH (luteinizing hormone) and T (testosterone) levels of F1 adult male rats were determined using enzyme-linked immunosorbent assay kits (R&D Systems, Inc., Minneapolis, MO, USA) according to the manufacturer's recommended protocols.

2.4. Quantification of ZEN and its metabolites

The preliminary treatment was conducted according to the procedures outlined by (Koraichi et al., 2012). ZEN and metabolites concentrations were determined by high performance liquid chromatography (HPLC) according to (Zöllner et al., 2002) and (Koraichi et al., 2012) with modifications.

2.5. Total RNA extraction and real-time quantitative PCR

Total mRNA was extracted from tissues with Trizol® (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. The quality and concentration of RNA were estimated by nucleic acid concentration analyzer (NanoDrop 2000; Thermo Fisher, Waltham, MA, USA). 1 μg of total RNA was reverse-transcribed in a 20 μL reaction using a PrimeScript™ RT reagent Kit (Takara, DRR037A). cDNA was stored at −20 °C until use in real-time quantitative PCR.

Expression levels of 7 genes: Gonadotropin-releasing hormone receptor (GnRHr), estrogen receptor alpha (Esr1), 3β-hydroxysteroid dehydrogenases (3β-HSD), steroidogenic acute regulatory protein (StAR) and ATP Binding Cassette Transporters b1 (ABCb1), ABCc1, ABCc5 were analyzed using Real-time q-PCR (CFX384, Bio-Rad) as described by (Huang et al., 2015). The primer sequences used are presented in (Supplementary Table 1). A 2^{-ddCt} method was used for the quantification with glyceraldehyde 3-phosphate dehydrogenase