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Atrazine triggers developmental abnormality of ovary and oviduct in quails (*Coturnix Coturnix coturnix*) via disruption of hypothalamo-pituitary-ovarian axis

Lei Qin [a], Zheng-Hai Du [a], Shi-Yong Zhu [a], Xue-Nan Li [a], Nan Li [b], Jing-Ao Guo [a], Jin-Long Li [a, *], Ying Zhang [c, **]

[a] College of Veterinary Medicine, Northeast Agricultural University, Harbin, 150030, PR China
[b] National Research Insitiute for Family Planning, Beijing, 100081, PR China
[c] School of Resources & Environment, Northeast Agricultural University, Harbin, 150030, PR China

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ABSTRACT

There has been a gradual increase in production and consumption of atrazine (ATR) in agriculture to meet the population rising demands. Female reproduction is necessary for growth and maintenance of population. However, ATR impact on females and particularly ovarian developmental toxicity is less clear. The aim of this study was to define the pathways by which ATR exerted toxic effects on ovarian development of ovary and hypothalamo-pituitary-ovarian (HPO) axis. Female quails were dosed by oral gavage from sexual immaturity to maturity with 0, 50, 250 and 500 mg ATR/kg/d for 45 days. ATR had no effect on mortality but depressed feed intake and growth and influenced the biochemical parameters. Notably, the arrested development of ovaries and oviducts were observed in ATR-exposed quails. The circulating concentrations of E2, P, LH and PRL were unregulated and FSH and T was downregulated in ATR-treated quails. The mRNA expression of GnRH in hypothalamo and LH in pituitary and FSH in ovary was downregulated significantly by ATR exposure and FSH and PRL in pituitary were upregulated. ATR exposure upregulated the level of P450scc, P450arom, 3β-HSD and 17β-HSD in ovary and downregulated ERβ expression in female quails. However, ATR did not change ERα expression in ovary. This study provides new insights regarding female productive toxicology of ATR exposure. Ovary and oviduct in sexually maturing females were target organs of ATR-induced developmental toxicity. We propose that ATR-induced developmental abnormality of ovary and oviduct is associated with disruption of gonadal hormone balance and HPO axis in female quails.

1. Introduction

The triazine herbicide ATR is widely used in agricultural to control the unwanted growth of grasses and broadleaf weeds. Being one of the most commonly used pesticides in the world (Hayes et al., 2010), ATR is widespread in the environment and a frequently detected contaminant in waterways. Like BPA and other chemicals, there are scientific indications that ATR has endocrine-disrupting potential (Hayes et al., 2010; Jin et al., 2014; Thompson et al., 2015), causing mammary gland tumors in rodents (Cooper et al., 2007), and altering male reproduction (Stanko et al., 2010). Despite ATR has been banned in European Union and been restricted in other countries, it is still being used in large quantities worldwide up to now. It is one of the most widely used agricultural pesticides in United States (Barr et al., 2007), and its application in Asian countries has been growing. There has been a gradual increase in production and consumption of ATR in agriculture to meet the population rising demands. Female reproduction is necessary for growth and maintenance of population. Therefore, humans and wildlife are at risk for exposure to ATR. ATR has been considered as an endocrine disruptor due to alterations caused on hormone-regulated systems in various taxa (De La Casa-Resino et al., 2012; Hayes et al., 2011; McMullin et al., 2004; Salaberria et al., 2009). However, little is known about its effects on the development of reproductive organ in females.

* Corresponding author.
** Corresponding author.
E-mail addresses: Jinlongli@neau.edu.cn (J.-L. Li), zhangyinghr@hotmail.com (Y. Zhang).

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In recent decades, there has been an increasing concern on clarifying the toxicological mechanisms of environmental chemicals to cause alterations in the reproductive system of humans and animals. ATR like other herbicides induces endocrine disruption and consequently interferes with various hormones physiological functions. Numerous reports have suggested that ATR might have adverse effects on the reproductive function (Cooper et al., 2007; Feyzi-dehkhargani et al., 2012; Friedmann, 2002; Kniewald et al., 2000; Stoker et al., 1999; Swan, 2006; Solomon et al., 2008; Trentacoste et al., 2001). In the male offspring a slower maturation of gonadotrophic system due to chronic expoxure to ATR was observed. In female, it has been shown that ATR could inhibit the development of ovary and oviduct in rats and cause infertility (Goldman et al., 2013; Zhao et al., 2014). It has also been reported that ATR could induce endocrine disruption and consequently interfere with physiological functions of various hormones (Jin et al., 2014; Weber et al., 2013). Consistent with these observations, Friedmann (2002) demonstrated that ATR had significantly reduced the serum and testicular testosterone (T) levels, both in acute toxicity test and chronic toxicity test, in juvenile Sprague–Dawley male rats by gavage. A recent study demonstrated that the serum levels of T, follicle stimulating hormone (FSH), luteinizing hormone (LH), and inhibin-B (INH–B) had decreased by 85% after 48 d exposure to high dose (300 mg/kg BW/day) of ATR in adult male Wister rats (Feyzi-dehkhargani et al., 2012). However, there is still little knowledge on the reproductive and developmental toxicity in female animals induced by ATR.

It is well known that ATR may impact endocrine activity and notably alter androgen/estrogen balance. ATR is a serious health concern, and numerous studies have been devoted to studying the effects of ATR on steroidogenic enzymes influenced steroid secretion and thus lead to reproductive toxicity (Basini et al., 2012; Payne and Youngblood, 1995; Pogrmic et al., 2009; Pogrmic-Majkic et al., 2010; Quignot et al., 2012b). In females, sex steroids are synthesized primarily in the ovaries and derived from cholesterol through a series of biochemical reactions (Foradori et al., 2013; Goldman et al., 2013; Henare et al., 2012; McMullin et al., 2004). However, the effects of ATR on the development of ovary and oviduct and the tuned balance between estrogens and androgens are not yet well clear.

Assessing ATR reproductive toxicity in female animals is a challenge, given the complexity of the endocrine system and despite the increasing development of data on its workings. To explore the effects and mechanism of hormonal balance disruption and the developmental abnormality of ovary and oviduct caused by ATR, female quails (*Coturnix Coturnix coturnix*) were employed as the experimental model orally given ATR daily from sexual immaturity to maturity. The aim of this study was to define the pathways by which ATR exerted the effects on the hypothalamo-pituitary-ovarian (HPO) axis and the developmental of ovary and oviduct, clarify the mechanism of ATR-induced toxicity in female animals.

2. Materials and methods

2.1. Animals and treatments

Female European quail (*Coturnix C. coturnix*) chicks aged 18 days and weighted 86.7 ± 6.4 g were purchased from Wan Jia farm in Harbin, China. Chemical ATR ($C_8H_{14}ClN_5$, CAS: 1912-24-9, ≥90% purity) was from Zhonghe Chemical Limited Company (Binzhou, China). Birds were housed in cages in an environmentally controlled room (temperature 24–28 °C and fluorescent lights provided a photoperiod of 12 h light and 12 h dark). Feed and water were offered ad libitum during the experiment. The birds were administered ATR once a day orally by gavage for 45 days. The gavage volume was 0.5 mL and the amount of gavage was adjusted everyday according to the varied weight of each quail. After one-week acclimation, the quails were randomly divided into four groups i.e., Group 1 (Control) treated with 0 mg/kg BW/day ATR, Group 2 (50 mg/kg ATR) treated with 50 mg/kg BW/day ATR, Group 3 (250 mg/kg ATR) treated with 250 mg/kg BW/day ATR, Group 4 (500 mg/kg ATR) treated with 500 mg/kg BW/day ATR. The ATR dose employed in the present investigation was chosen on basis of recent studies by Hussain et al. (2011) and Wilhelms et al. (2005). The current ecological risk assessment for ATR in avian species established by the USEPA reports a dietary LOAEL (lowest observable adverse effect level) of 675 mg/kg in the northern bobwhite quail, ATR exhibits modest reproductive toxicity in female birds (USEPA, 2002). In this study, all the experiments conducted in animals were in accordance with the guidance of ethical committee for research on laboratory animals.

The birds were monitored daily for clinical signs and total body weights gains. At the end of the experiment, birds were fasted before the day of sacrifice, and their hypothalamo, pituitary, ovary and oviduct were carefully dissected out. The blood was collected from the heart of each bird and centrifuged at 3000 rpm for 10 min to obtain the serum. The serum were divided into two portions, one for biochemical analyses and a second to be stored at −80 °C for assays.

2.2. Determination of biochemical parameters

Blood samples were used to investigate changes in the serum enzymes and concentration of ions considered to be biochemical indicators of hepatobiliary, renal and myocardial enzyme. Serum Ca, Mg, P and glucose (Glu) concentrations were measured. Both the activities of creatine kinase (CK), lactate dehydrogenase (LDH), choline esterase (CHE), contents of creatinine (Cre), blood urea nitrogen (BUN), total protein (TP), albumin (ALB), total bilirubin (T.BILT) and direct bilirubin (D.BILT) were measured. The activities or contents of biochemical parameters were detected using the detection kits (Jiangsu SINNOWA Medical Technology Company, China) by a biochemical auto-analyzer.

2.3. Histopathological studies

Oviducts and ovaries were washed in cold saline and soak dried on filter paper. A portion of organ was fixed in 10% buffered formalin and embedded in paraffin. Sections of 5 μm thickness were cut and stained with hematoxylin and eosin for microscopic examination.

2.4. Hormone analysis

To identify ATR-induced changes in circulating concentrations of reproductive hormones, serum concentrations of E_2, P, FSH, LH, PRL and T were determined. All six reproductive hormone were determined using ^{125}I Radioimmunoassay (RIA) Kits (HAT CO. LTD. China) according to the manufacturer's protocol. Radioactivity was determined using an automatic gamma counter. All samples were run in duplicate in a single assay to avoid interassay variation.

2.5. RNA purification and quantitative real-time PCR

Total mRNA was extracted from hypothalamo, pituitary and ovary using RNAout reagent (Beijing Tiandz, Inc. China), according to the manufacturer's instructions. First cDNA strand was synthesized using Oligo (dT) primers and Transcript Reverse Transcriptase (Beijing TransGen Biotech Co. Ltd., China). The primers for real-time amplification of relative cDNAs were designed using Oligo 7.22