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Oral exposure to atrazine modulates hormone synthesis and the transcription of steroidogenic genes in male peripubertal mice

Yuanxiang Jin, Linggang Wang, Zhengwei Fu *

College of Biological and Environmental Engineering, Zhejiang University of Technology, Hangzhou 310032, China

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ABSTRACT

Atrazine (ATZ) is a widely used herbicide and is considered an endocrine disruptor of different organisms. However, the molecular interactions of ATZ with biological targets in mammalian endocrine systems are not understood fully. In the present study, we observed that ATZ administration (50, 100 and 200 mg/kg) for 3 weeks to peripubertal male ICR mice exerted adverse effects on several physiological features; these effects included a significant decrease in the body and liver weights and an increase in the relative testis weight. In addition, the serum testosterone (T) concentration was significantly decreased in all ATZ-treated mice, and the serum estradiol (E2) concentration and aromatase activity were significantly increased in mice exposed to 100 and 200 mg/kg ATZ. These results suggest that ATZ exposure affected hormone homeostasis in male mice. We also found that the transcript levels of the steroidogenic enzyme genes *p450scc, p450 17α1* and *17β-HSD* were significantly reduced in the testes of mice exposed to 100 and 200 mg/kg ATZ for 3 weeks. Given the results of the present study and previous reports, it is possible that ATZ reduces the T concentration in peripubertal male mice by affecting the transcription of steroidogenic genes, such as *p450scc*, *p450 17a1* and *17β-HSD*. This study provides new insights into the mammalian toxicological mechanism of ATZ.

1. Introduction

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine, ATZ) is a triazine herbicide that effectively inhibits photosynthesis in broadleaf weeds and grasses (Protection Agency. et al., 2003). ATZ is primarily applied to increase the yields of food crops, such as soy, maize and sugarcane, and has been the most commonly used pesticide in the United States and other countries for more than 50 years. In China, where ATZ is used as a broad-spectrum herbicide applied to major field crops such as corn, wheat and soybeans, the estimated current annual usage of ATZ for field applications has been reported to be several million kilograms (Deng et al., 2005; Jin et al., 2010; Song et al., 2009). The widespread use of ATZ has resulted in contamination in several countries, including China (Gfrerer et al., 2002; Na et al., 2006; Ren et al., 2002; Ye et al., 2001).

Currently, endocrine disruption induced by different chemicals is among the most important subjects in mammalian toxicology (Badraoui et al., 2010; Schug et al., 2011; Yuan et al., 2009). As a widely used herbicide, ATZ has been reported to disrupt several endpoints of the endocrine systems of different organisms. For example, ATZ exposure reduced the number and motility of sperm in male mammals (Abarikwu et al., 2010; Kniewald et al., 2000). Recently, Victor-Costa et al. Victor-Costa et al. (2010) reported that ATZ doses higher than 50 mg/kg decreased body weights, increased adrenal weights and transiently increased testis weights followed by testis atrophy. A number of previous studies also indicated that ATZ had the potential to decrease the testosterone (T) levels in mammals (Friedmann, 2002; Trentacoste et al., 2001; Victor-Costa et al., 2010), reptiles (Crain et al., 1997), amphibians (Hayes et al., 2002) and fishes (Spano et al., 2004), indicating that ATZ affects hormone homeostasis in several species of animals. Several studies have indicated that ATZ could increase the transcription of *cyp19a1* (Fan et al., 2007) and the activity of aromatase (Hayes et al., 2002; Heneweer et al., 2004; Sanderson et al., 2000), which is the enzyme responsible for catalyzing the conversion of T to estradiol (E2). These results might possibly explain the increased E2 production and concomitant decreased T levels in certain organisms exposed to ATZ. On the contrary, a series of studies reported that ATZ did not affect the gonadal aromatase activity in male animals (Crain et al., 1999; Hecker et al., 2005; Kazeto et al., 2004). More recently, Victor-Costa et al. Victor-Costa et al. (2010) reported that 3β-hydroxysteroid dehydrogenase (3β-HSD) inhibition might represent an alternative mechanism through which ATZ affected the testicular androgenesis and spermatogenesis in adult rats. Thus, despite growing concerns over the toxicological effects of ATZ, its molecular interactions with biological targets in mammalian endocrine systems are not fully understood. In particular, there is currently little evidence establishing the effects of ATZ on androgen-estrogen homeostasis

* Corresponding author. Fax: +86 571 8832 0599.
E-mail address: azwfu2003@yahoo.com.cn (Z. Fu).

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and the expression patterns of the main steroidogenic genes in peripubertal male mice.

As demonstrated by previous studies, it is clear that ATZ influences the mammalian endocrine system; however, the molecular mechanisms mediating these effects are still not clearly understood, especially in adolescent organisms. Thus, in the present study, we first examined the serum sexual hormone levels and aromatase activity in peripubertal male mice after exposure to a range of ATZ doses for 3 weeks to understand ATZ-induced endocrine disruption better in adolescent mice. Next, the expression patterns of genes involved in cholesterol synthesis, including *HMG-CoA synthase* and *HMG-CoA reductase*, were determined in the liver and testis. Finally, the expressions of genes involved in cholesterol transport, T synthesis, and T to E2 conversion were also examined in the testis to elucidate the potential mechanism through which ATZ disrupts the endocrine system. In the present study, we evaluated the effects of ATZ on male mice at the physiological and transcriptional levels to provide new insights into the mammalian toxicological mechanism of ATZ.

2. Material and methods

2.1. Animals and experimental design

ATZ (CAS No.: 1912–24-9; purity:>97%) was purchased from ICI and used as received. Twenty-eight three-week old peripubertal male ICR mice (*Mus musculus*) were purchased from the China National Laboratory Animal Resource Center (Sungkiang, Shanghai, China). The mice were kept in animal facilities (illumination with strip lights, 200 lux at the cage level; 22 ± 1 °C), and water and food were available *ad libitum*. After one week, the mice were randomly divided into 4 groups. Next, the four groups of young male mice were orally administered ATZ (0, 50, 100 or 200 mg/kg) daily for three weeks. The body weight of each mouse was recorded every other day. Differences in body weights were not observed among these four groups at the beginning of the experiment. The animals were maintained under normal facility conditions (lights on at eight o'clock, lights off at twenty o'clock; illumination with strip lights, 200 lux at the cage level; 22 ± 1 °C). Water was available *ad libitum* during entire treatment period, and food was available *ad libitum* only at night during entire treatment period. All the mice were sacrificed after anesthetization with ether on the last day of treatment. Blood sera were collected for hormone measurements. The livers and testes were quickly removed and weighed, after which the tissues were immediately frozen in liquid nitrogen and stored at −80 °C until use. Every effort was made to minimize animal suffering in each experiment. All experiments were performed in accordance with the Guiding Principles in the Use of Animals in Toxicology.

2.2. Determination of serum hormone concentrations

Serum was separated by centrifugation (5000 rpm for 5 min) and stored at −20 °C until the assays for measuring the T and E2 concentrations and the aromatase activity were performed. The level of T and E2 or the activity of aromatase in 10 μL of serum from each sample was determined by the corresponding mouse ELISA kits (RapidBio, USA) according to the manufacturer's instruction. All of the ELISA assays were measured in a 96-well micro well plate using a microplate reader (Power wave XS, Bio-TEK, USA).

2.3. Gene expression analysis

Total RNA was isolated from the livers and testes of mice using a TRIzol reagent (Takara Biochemicals, China) according to the manufacturer's protocol. After denaturalization, RNA was used to synthesize cDNA using a reverse transcriptase kit (Toyobo, Tokyo, Japan). A 1-μL aliquot of each RT product or diluent was used for real-time quantitative polymerase chain reaction (RT-qPCR). RT-qPCR was performed on an Eppendorf MasterCycler® ep RealPlex^4 (Wesseling-Berzdorf, Germany). Oligonucleotide primers were used to detect the expression of glyceraldehyde-3-phosphate dehydrogenase *(GAPDH),* 3-hydroxy-3-methyl-glutaryl-CoA synthase *(HMG-CoA synthase),* 3-hydroxy-3-methyl-glutaryl-CoA reductase *(HMG-CoA reductase),* low-density lipoprotein receptor *(LDL-R),* scavenger receptor class B type 1*(SR-B1),* peripheral benzodiazepine receptor *(PBR),* steroidogenic acute regulatory protein *(StAR),* cytochrome P450 cholesterol side-chain cleavage enzyme *(P450scc),* 3β-hydroxysteroid dehydrogenase *(3β-HSD),* cytochrome P450 17α-hydroxysteroid dehydrogenase *(P450 17α)* and 17β-hydroxysteroid dehydrogenase *(17β-HSD)* using the SYBR Green system (Toyobo, Tokyo, Japan). Detailed information regarding the primers is presented in Table 1. The *GAPDH* transcript was used as the house-keeping gene. The following PCR protocol was used with the Eppendorf MasterCycler® ep realPlex^4 (Wesseling-Berzdorf, Germany): denaturation for 1 min at 95 °C followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C. The PCR protocol and relative quantification of gene expression among the treatment groups were analyzed according to our previous report (Jin et al., 2011).

2.4. Data analysis

Values are expressed as the mean ± SEM. Data were evaluated by one-way ANOVA followed by Duncan Significant Difference test using SPSS 13.0 (SPSS, Chicago, IL, USA). Differences with p-values < 0.05 were considered to be significant.

Table 1
Sequences of primer pairs used in the real-time quantitative PCR reactions.

Gene name	Primer sequences	Accession number
GAPDH	Forward 5′-AGAACATCATCCCTGCATCCA-3′ Reverse 5′-CCGTTCAGCTCTGGGATGAC-3′	NM_008084.2
HMG-CoA synthase	Forward 5′-TGTGGCACCGGATGTCTTT-3′ Reverse 5′-GACCAGATACCACGTTCCTTCAA -3′	NM_145942.4
HMG-CoA reductase	Forward 5′-TGTGGTTTGTGAAGCCGTCAT-3′ Reverse 5′-CGTCAACCATAGCTTCCGTAGTT-3′	NM_008255.2
SR-B1	Forward 5′-CCCTTCGTGCATTTTCTCAAC-3′ Reverse 5′-CATCCCAACAAACAGGCCA -3′	BC004656
LDL-R	Forward 5′-GGAAAATGCATCGCTAGCAAGT-3′ Reverse 5′-ATTGGACTGACAGGTGACAGACA-3′	AF425607.1
PBR	Forward 5′-AGTTCGTGGCACTGCATAAGC-3′ Reverse 5′-GCTGCCCATTCTCTCCTCCTA-3′	D21207.1
StAR	Forward 5′-AAGGAAAGCCAGCAGGAGAAC-3′ Reverse 5′-TCCATGCGGTCCACAAGTT-3′	BC082283.1
P450scc	Forward 5′-CCATCAGATGCAGAGTTTCCAA-3′ Reverse 5′-TGAGAAGAGTATCGACGCATCCT -3′	AF195119.1
3β-HSD	Forward 5′-GGAGGCCTGTGTTCAAGCAA-3′ Reverse 5′-GGCCCTGCAACATCAACTG-3′	NM_008293.3
P450 17a	Forward 5′-CCATCCCGAAGGACACACAT-3′ Reverse 5′-CTGGCTGGTCCCATTCATTT -3′	NM_007809.3
17β-HSD	Forward 5′- AGGACCTGGATTCAGTTCCAAGC-3′ Reverse 5′- CTAGGTGTTTGAGAAAGTCTG-3′	NM_198030
ERα	Forward 5′-AGCTCAACAGCGTGTCGCCT-3′ Reverse 5′-TGCACACGGCACAGTAGTGA-3′	NM_007956
ERβ	Forward 5′-AGCTGGTGCTCACCCTGCTG-3′ Reverse 5′-GATGAGCTTGCCGGGGTGGT-3′	BC145329
AR	Forward 5′-CGGTGCGTCCCACTCCTTGT-3′ Reverse 5′-TGCCAGAGCACCCCAAGCTC-3′	NM_013476