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Exposure of mice to atrazine and its metabolite diaminochlorotriazine elicits oxidative stress and endocrine disruption

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ARTICLE INFO

Article history:
Received 19 August 2013
Received in revised form
17 February 2014
Accepted 17 February 2014
Available online 26 February 2014

Keywords:
Atrazine
Diaminochlorotriazine
Oxidative stress
Endocrine disruption
Mice

ABSTRACT

Effects of atrazine (ATZ) and its metabolite diaminochlorotriazine (DACT) on the induction of oxidative stress and endocrine disruption were studied in mice. Body and liver weights decreased in all ATZ and DACT treated groups. Hepatic activities of superoxide dismutase (SOD) increased significantly after 1 week of intraperitoneal injection of 200 mg/kg ATZ, 100 and 200 mg/kg DACT. Hepatic activities of catalase (CAT) and glutathione S-transferase (GST) were also affected by the treatment with 200 mg/kg DACT. In serum, the glutathione peroxidase (GPX) and GST activities and glutathione (GSH) content decreased significantly in the 200 mg/kg DACT treated group. Moreover, the administration of ATZ and DACT decreased the transcription levels of key genes related to cholesterol transport and testosterone (T) synthesis including *scavenger receptor class B type 1 (SR-B1)*, *cytochrome P450 cholesterol side-chain cleavage enzyme (P450scc)* and *cytochrome P450 17α-hydroxysteroid dehydrogenase (P450 17α)* in testes. Furthermore, the treatment with 200 mg/kg DACT significantly decreased the serum and testicular T levels, while the treatment with 200 mg/kg ATZ significantly decreased the testicular T levels. The results indicated that the acute exposure to ATZ and DACT induced oxidative stress and endocrine disruption in mice, and DACT showed much more toxic than ATZ did.

1. Introduction

As a wild used herbicide, atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine, ATZ) effectively inhibits photosynthesis in broadleaf weeds and grasses (EPA, 2003). ATZ is primarily applied to increase the yield of food crops such as soy, maize and sugarcane in many countries for over the past half-century. As reported, approximately 64–80 million pounds of ATZ are applied annually in the United States for agricultural and residential purposes; this amount has remained relatively constant over the past few decades (Kiely et al., 2004; Barr et al., 2007). As a result, ATZ is the most commonly detected herbicide in ground and surface waters (Gaynor et al., 2002; Shaw et al., 2010), and its concentration even reaches 1600 ng/l in Liao-He river in China (Gfrerer et al., 2002).

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http://dx.doi.org/10.1016/j.etap.2014.02.014
1382-6689/

Currently, oxidative stress and endocrine disruption are among the most important subjects in environment chemicals toxicology (López et al., 2007; Badraoui et al., 2010; Mansour and Mossa, 2010), as herbicides including ATZ may be directly involved in these processes. Growing evidence proved that the herbicide of ATZ had the potential to induce oxidative stress (Jin et al., 2010; Pogrmic-Majkic et al., 2012; Xing et al., 2012; Blahová et al., 2013), DNA damage (Song et al., 2009) and endocrine disruption (Oka et al., 2008; Salaberria et al., 2009; Jin et al., 2013) in different organisms. In fact, ATZ can enter into the wildlife and human *via* several different pathways. In mammalian, a number of studies indicated that ATZ was metabolized primarily by cytochromes P450 and, to a much lesser extent, by glutathione transferases (GSTs), to several different metabolized products including desethyl atrazine (DE), desisopropyl atrazine (DIP), diaminochlorotriazine (DACT), atrazine-glutathione (ATZ-SG) and atrazine-mercapturate (ATZ-mercap) (Adams et al., 1990; Hanioka et al., 1999; Ross et al., 2009). Among these products, DACT is the major *in vivo* metabolite of ATR detected in mice and rat plasma, urine and tissues (Brzezicki et al., 2003; Ross and Filipov, 2006; Ross et al., 2009). Recently, some evidence also indicated that DACT was the most frequently detected urinary metabolite of ATR in humans (Barr et al., 2007). However, whether the DACT has the potential to influence mammalian anti-oxidative and endocrine systems still remains unclear.

The objective of the present study is to compare the effects on oxidative stress and endocrine disruption induced by ATZ and DACT in male mice. We firstly examined the status of the anti-oxidants enzymes in the liver and serum in male mice after exposed to various doses of ATZ and DACT for one week. Then, the expression patterns of genes related to cholesterol transport and T synthesis, and the serum and testicular T levels were also examined to elucidate the potential mechanism of endocrine disruption induced by ATZ and DACT. This study is intended to provide new insights into the mammalian toxicological mechanism of ATZ and its main metabolite DACT.

2. Materials and methods

2.1. Animals and experimental design

ATZ (CAS No.: 1912-24-9; purity: >97%) was purchased from TCI and DACT (CAS No.: 3397-62-4; purity: >95%) was purchased from Sigma–Aldrich, and both of the chemicals used as received. Totally 30 six-week old male ICR mice (*Mus musculus*) were purchased from the China National Laboratory Animal Resource Center (Shanghai, China). All the mice were kept in animal facilities (illumination with strip lights, 200 lx at cage level; 22 ± 1 °C). Water and food were available *ad libitum*. After one week, all the mice were divided into 5 groups randomly. Then, the five groups were intraperitoneally injected by ATZ and DACT (0, 100 and 200 mg/kg/bodyweight, respectively) every other day for one week. And totally 4 times injections were conducted (day 1, day 3, day 5 and day 7) according to the body weight. The control group was injected with the same volume of corn oil (Wako, Japan). During the treatment, the animals were maintained under normal facilities (light-on at eight o'clock, light-off at twenty o'clock; illumination with strip lights, 200 lx at cage level; 22 ± 1 °C). Water was available *ad libitum* during whole period of the treatment, while food was available only at night during whole period of the treatment. Finally, at day 8, all of 30 mice were sacrificed. The mice were anesthetized by ether before sacrificing. Livers and testes were quickly removed and weighed, after which they were immediately frozen in liquid nitrogen and kept at −80 °C until use. Blood sera were also collected for further measurement. 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 the activities of SOD, CAT, GST, SOD and GSH content in the liver and serum

Livers were defrosted and homogenized with 10 volumes of cold buffer consisting of 250 mmol L^{-1} sucrose, 5 mmol L^{-1} Tris–HCL and 0.1 mmol L^{-1} EDTA-2Na (pH 7.5). The homogenate was centrifuged at 4000 × *g* at 4 °C for 15 min to obtain the supernatant for the enzyme activity assays. Serum was separated by centrifugation (5000 rpm for 5 min) and stored at −20 °C. The activities of SOD, CAT, GPX and GST in the liver and serum were determined using kits purchased from the Nanjing Jianchen Institute of Biotechnology (Nanjing, China) according to the manufacturer's instructions and our previous reports (Jin et al., 2010, 2011). Protein concentration in each sample was determined using the BCA protein assay kit provided by Sangon Biotech (Shanghai, China). All the measurements were made on a microplate reader (Power wave XS, Bio-TEK, USA) according to the manufacturer's instructions.

2.3. Gene expression analysis

Total RNA was isolated from the testes of mice using 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).Real-time quantitative polymerase chain reaction (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*), 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*) genes using the SYBR Green system (Toyobo, Tokyo, Japan). The *GAPDH* transcript was used as a house keeping gene in the present study. The detail information of main primers was indicated in our previous reports (Jin et al., 2012, 2013). The PCR protocol is: 1 min at 95 °C for denaturation, followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C. The relative quantification of genes expression among the treatment groups was