Acta Histochemica 118 (2016) 456–463

Contents lists available at ScienceDirect

Acta Histochemica

journal homepage: www.elsevier.de/acthis

Protective role of garlic oil against oxidative damage induced by furan exposure from weaning through adulthood in adult rat testis

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ARTICLE INFO

Article history:
Received 3 March 2016
Received in revised form 21 April 2016
Accepted 22 April 2016

Keywords:
Furan
Rat testis
Oxidative stress
Histopathology
Caspase-3
CYP2E1

ABSTRACT

Furan is produced in a wide variety of heat-treated foods via thermal degradation. Furan contamination is found to be relatively high in processed baby foods, cereal products, fruits juices, and canned vegetables. Several studies have demonstrated that furan is a potent hepatotoxin and hepatocarcinogen in rodents. However, few studies have investigated the toxic effects of furan in the testis. In addition, the exact mechanism(s) by which furan exerts toxicity in the testis has not been fully elucidated. In this study, we investigated the potential of furan exposure from weaning through adulthood to induce oxidative stress in adult rat testis, as well as the potential of garlic oil (GO) to ameliorate the induced toxicity. Our results reveal that furan administration significantly reduced serum testosterone levels and increased the levels of malondialdehyde (MDA); furthermore, furan administration decreased significantly the enzymatic activity of testicular antioxidants, including glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) and induced histopathological alterations in the testis. GO co-administration ameliorated the reduction in testosterone levels and dramatically attenuated the furan-induced oxidative and histopathological changes. In addition, Go significantly down-regulated the increased caspase-3 and cytochrome P450 2E1 (CYP2E1) expression in the furan-treated testis. To the best of our knowledge, this study is the first to demonstrate the furan-induced oxidative changes in the adult rat testis and the protective role of GO to ameliorate these changes through its antioxidant effects and its ability to inhibit CYP2E1 production.

1. Introduction

Heat-induced food contaminants, including furan, have attracted considerable attention recently (Becalski et al., 2010; Terrell et al., 2014; Churchwell et al., 2015). Surveys performed by various health agencies, including the U.S. Food and Drug Administration (FDA, 2007), Health Canada (Becalski et al., 2010), the European Food Safety Authority (EFSA, 2011), and the Swiss Office of Public Health (Zoller et al., 2007), have reported that furan is present in a wide range of foods, including roasted coffee, baby foods, cereal products, fruits juices, canned vegetables, cooked and canned meats, beer, and wheat breads. The FDA estimated human exposure to furan according to concentration data posted through spring 2007 and reported a mean and 90th percentile of 0.26 and 0.61 μg furan/kg-bw/day for the 2+-year-olds from the consumption of adult food, and 0.41 and 0.99 μg furan/kg-bw/day for the 0- to 1-year-olds from the consumption of infant foods (Dinovi and Mihalov, 2007).

The EFSA reported that brewed coffee was the major contributor to furan exposure for adults, while the major contributors to exposure in toddlers and other children were fruit juice, milk-based products, and cereal-based products. In addition for toddlers and infants, jarred baby foods were among the major sources of exposure (EFSA, 2011). This finding is of particular concern because infants are particularly vulnerable to carcinogens. In addition, the long-term effects of furan on the health of children has not been fully studied (Bakhiya and Appel 2010; Scholl et al., 2013).

Because furan is carcinogenic to rodents and there is widespread human exposure, it has been classified as a potential human carcinogen (Peterson et al., 2000; Moro et al., 2012; Mariotti et al., 2013; Kim et al., 2015). Several studies have demonstrated the toxic effects of furan administration on liver and kidneys of rodents (Selmanoglu et al., 2012; Webster et al., 2014; Gill et al., 2014). However, few studies have investigated the effect of furan on the testis (Karacaoglu and Selmanoglu, 2010; Gill et al., 2010; Cooke et al., 2014). In addition, the exact mechanism(s) by which furan exerts toxicity in the testis has not been fully elucidated. Under-

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http://dx.doi.org/10.1016/j.acthis.2016.04.008
0065-1281/

standing the underlying mechanism of furan toxicity is critical for identifying the appropriate protective applications.

The cytochrome P450 (CYP) enzymes are a class of heme-containing enzymes implicated in phase I metabolism of a wide variety of chemicals (Hrycay and Bandiera, 2015). The CYP family member, CYP2E1, is documented to play a role in the bioactivation of many xenobiotics, pro-carcinogens, and environmental pollutants, such as furan and acrylamide (Ghanayem and Hoffler, 2007; Gates et al., 2012; Khedhaier et al., 2008; Ioannides, 2013; Raunio et al., 2015). CYP2E1 is not only expressed in the liver but in many other tissues, including the kidney, the lung, the pancreas, the brain, and the testis (Healy et al., 1999; Arinc et al., 2007; Gonzalez, 2005; Zhong et al., 2012; Leung et al., 2013). CYP2E1 is known to be involved in the generation of the reactive oxygen species (ROS) (Robertson et al., 2001; Lewis, 2001; Gonzalez, 2005; Hickling et al., 2010; Zhong et al., 2012; Leung et al., 2013; Valencia-Olvera et al., 2014). Therefore, CYP2E1 in the testis may indicate the in situ metabolic activation of environmental pollutants, such as furan, and the subsequent induction of oxidative stress (Shayakhmetova et al., 2013; Rajeh et al., 2014; Shayakhmetova et al., 2015).

A substantial body of evidence has demonstrated garlic and its organosulfur compounds are powerful and potent antioxidants through their radical-scavenging functions and ability to modulate antioxidant enzyme defence systems (Park et al., 2009; Srinivasan, 2014). In addition, garlic modulates several phase I detoxifying enzymes, such as CYP enzymes, which may be linked to its protective activity against carcinogenicity and against formation of genotoxic oxidative metabolites from xenobiotics (Premdas et al., 2000; Wargovich, 2006; Zeng et al., 2009; Tsai et al., 2012; Zhang et al., 2013). A decrease in CYP2E1 activity and protein expression has been demonstrated in rats fed a diet containing 5% garlic powder (Le Bon et al., 2003). The formation of glycidamide, an active metabolite of acrylamide, was down-regulated in the liver as a result of the inhibition of CYP2E1 by garlic ingredients (Taubert et al., 2006). Taken together, these data suggest that garlic can be a potential beneficial candidate in preventing furan-induced oxidative stress.

The present study presents, to the best of our knowledge, the first investigation of the potential of furan to induce oxidative stress damage in the testis of adult rats following exposure from weaning through adulthood. In addition, we also evaluated the possible protective effect of garlic oil against furan toxicity.

2. Methodology

2.1. Animals

Male Sprague-Dawley rats (aged 3–4 weeks old) were maintained in the animal house of the Research Institute of Ophthalmology, Giza, Egypt. The rats were subjected to a 12: 12-h light/dark cycle and allowed unlimited access to chow and water. All of the ethical protocols for animal treatment were followed and supervised by the animal facilities at the Research Institute of Ophthalmology, Giza, Egypt. All procedures involving the use of the rats were approved by The Animal Care and Use Committee, Faculty of Medicine, Menoufia University.

2.2. Experimental design

The rats were randomly divided into five groups; control, corn oil control (corn oil), garlic oil control (GO group), furan-treated (furan), and furan + GO-treated (furan + GO) (n = 10 per group). Animals in the control group were fed by standard food and water *ad libitum*. The corn oil group was gavage-fed with corn oil in an amount equal to that used in the furan treatment groups. In GO group, GO was administered orally by gavage at 80 mg/kg/day for 5 days per week for 90 days. Furan (CAS 110-00-9, Sigma-Aldrich Co. Ltd) was freshly dissolved in corn oil and was administered to rats orally via gavage at 4 mg/kg/day for 5 days per week for 90 days. The dose was chosen according to the 2-year NTP study (NTP, 1993). In the furan + GO-treated group, GO (El-Captain, Cairo, Egypt) was administered orally by gavage at 80 mg/kg/day 2 h before furan administration. There was no statistically significant difference between the control, corn oil, and GO groups; therefore, these groups were pooled into one group (control). At the end of the study, blood samples were collected from each rat. Rats were anaesthetized using ketamine (90 mg/kg) and xylazine (15 mg/kg) (i.p.) and decapitated. Dissected testes were processed for oxidative stress, histological, and immunohistological (IHC) assessments.

2.3. Testosterone hormone analysis

Blood samples taken for hormone analysis were centrifuged at 3500 rpm for 15 min. Serum testosterone levels were measured by the enzyme-linked immunosorbent assay (ELISA).

2.4. Assessment of oxidative stress and antioxidants indices

Testicular tissue (100 mg) was homogenized in 1 mL of phosphate buffer solution (PBS; pH 7.0) and the homogenate was used to measure MDA, GSH, SOD, and CAT levels using a spectrophotometer.

The extent of lipid peroxidation was assessed by measuring the MDA concentration (Wills, 1987). Trichloroacetic acid (TCA; 20%) was added to the homogenate and then centrifuged at 5000 rpm for 15 mins. The supernatant was collected and 0.5% thiobarbituric acid (TBA) solution was added. After boiling for 10 min in a water bath, the absorbance was measured at 532 nm. The concentration of MDA was calculated using the standard curve. The results were expressed as nanomoles (nmol) per milligram (mg) of protein.

Superoxide dismutase (SOD) activity was measured through the inhibition of nitroblue tetrazolium (NBT) reduction by O_2^- generated by the xanthine/xanthine oxidase system. The absorbance was measured at 550 nm. One SOD activity unit was defined as the enzyme amount necessary to cause 50% inhibition in 1 mL of reaction solution per milligram of tissue protein and the results were expressed as unit (U) per mg of protein (Misra and Fridovich, 1972).

Catalase (CAT) activity was detected using the ammonium molybdate method (Beers and Sizer, 1952) by measuring the intensity of a yellow complex formed by molybdate and H_2O_2 at 405 nm, after ammonium molybdate was added to terminate the H_2O_2 degradation reaction catalyzed by CAT. An enzyme activity unit was defined as the degradation of 1 millimole H_2O_2 per second per mg of tissue protein, and the enzyme activities were expressed as U/mg of protein.

Glutathione (GSH) level was determined according to the method of Ellman (1959). Dithiobis nitrobenzoate (DTNB) solution was added to the testicular homogenate and incubated for 1 h. The absorbance was measured at 412 nm. The concentration of GSH was calculated using the standard curve. The results were expressed as micromoles (μmol) per mg of protein.

2.5. Histological and immunohistological (IHC) study

Testes fixed in Bouin's solution and embedded in paraffin wax. For histological examination, 5-μm sections were deparaffinised and rehydrated using a graded ethanol (100%, 90%, and 70%) series and stained with haematoxylin and eosin (H&E).

For immunohistological staining, deparaffinised and rehydrated 5-μm sections were rinsed with PBS and blocked for 30 min in 0.1% H_2O_2, as an inhibitor of endogenous peroxidase activity. After