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Fluoride-induced oxidative stress is involved in the morphological damage and dysfunction of liver in female mice

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HIGHLIGHTS

- Fluoride causes morphological changes in liver tissue.
- Fluoride exposure damages ultrastructure in hepatocyte.
- Fluoride exposure increases the micronuclear rates of hepatocyte.
- Fluoride exposure disturbs hepatic markers homeostasis.
- Fluoride-induced oxidative stress leads to dysfunction of liver in female mice.

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ABSTRACT

Fluoride (F), one of the most toxic environmental and industrial pollutants, is known to exert hepatotoxicity. The contribution of oxidative stress to the F tolerance of liver remains largely unknown. In this study, the morphological and ultrastructural characteristics of liver were observed using hematoxylin and eosin staining and transmission electron microscopy (TEM), respectively. Oxidative-stress participations was analysed and the mRNA expression levels of catalase (Cat), glutathione peroxidase 1 (GSH-Px1), nitric oxide synthase 2 (NOS2), and superoxide dismutase 1 (SOD1) were investigated by real-time PCR. Changes in liver-function parameters were also detected. Results showed that the reactive content of reactive oxygen species increased significantly, whereas SOD and GSH-Px activities, as well as total anti-oxidising capability (T-AOC), decreased significantly, with increased nitric oxide (NO) and malondialdehyde (MDA) contents in liver and serum after 70 days of F treatment. The mRNA expression levels of Cat, GSH-Px1, and SOD were significantly downregulated, whereas NOS2 mRNA expression level was up upregulated, after F treatment for 70 days. Light microscopy also revealed that hepatocytes were fused into pieces; cell boundaries were unclear, and nuclei were lightly stained. TEM further showed that hepatocytes were characterised by vague nuclear and mitochondrial membranes, dilated endoplasmic reticulum, and aggravated vacuolar degeneration. Activities of alanine transaminase, aspartate aminotransferase, alkaline phosphatase and lactate dehydrogenase, as well as the level of total bilirubin in serum increased. Overall, these results indicated that F interfered with the balance of antioxidase activity and morphological changes in liver, which were involved in mouse liver dysfunction.

1. Introduction

Fluorine is one of the most widely distributed elements in nature and exists only in combination with other elements as fluoride (F), which is the main component of minerals in soil and rocks. A multi-generational investigation has shown that F is present in different tissues of humans and animals. In addition, F in low concentration is considered by some to be necessary for dental health (Chu and Lo, 2008; Murakami et al., 2009) and bone development (Chachra et al., 2010; Shim et al., 2011). The WHO has established that the upper intake level for F in drinking water is 1.5 mg/L to ensure the beneficial effects of F. However, among the 25 countries that have high F concentrations (>1.5 mg/L), such as China, India,

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México and Argentina, more than 200 million people suffer from endemic fluorosis (Amini et al., 2008). High concentrations of F have been proven harmful to the dental and biochemical parameters of experimental animals (Mandinic et al., 2010; Cardenas-Gonzalez et al., 2013; Perumal et al., 2013; Zhou et al., 2013; Sarkar et al., 2014).

F is an anion that can readily penetrate cell membranes through simple diffusion and under certain condition, and can virtually cause adverse effects on cell metabolism and function. Oxidative stress, one of the accepted mechanisms of F toxicity, is triggered by the imbalance between production and elimination of free radicals (Hassan and Yousef, 2009; Hassan and Abdel-Aziz, 2010; Agalakova and Gusev, 2012; Nabavi et al., 2012a). When the body antioxidant capacity can no longer protect the cell from oxidative damage, free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) accumulate and exert detrimental effects including lipid peroxidation (LPO), protein oxidation, and DNA damage (Ferreira et al., 2010; Kryston et al., 2011; Kubrak et al., 2011; Hilali et al., 2013; Smith et al., 2013). These detrimental effects can ultimately lead to changes in cell structure and function. To evaluate damage inflicted by oxidative stress, antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px), along with LPO products such as malondialdehyde (MDA), were studied as potential biomarkers (Zhang et al., 2007; Dogan et al., 2011; Liu et al., 2014; Malik et al., 2014). Previous studies have reported F-induced damage through oxidative stress in the brain, liver, kidney and other organs (Guo et al., 2003; Argüelles et al., 2004; Inkielewicz-Stepniak and Czarnowski, 2010; Nabavi et al., 2012b; Atmaca et al., 2014; Sarkar et al., 2014; Qin et al., 2015).

The liver is an important metabolic organ in the body, secreting bile and processing various nutrients into proteins. Most importantly, the liver functions in detoxifying tissue by transforming, neutralising, and eliminating toxins through hepatocyte-mediated enzymatic detoxification systems (Pereira et al., 2013). Many studies have shown that exposure to excessive F could induce damage to the liver, and the degree of damage is associated with the quantity of F ingestion (Xiong et al., 2007; Cao et al., 2013; Zlatković et al., 2014). Liver function tests work by determining the serum enzyme activities of alanine aminotransferases (ALT), aspartate aminotransferases (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) and the level of total bilirubin (TB), which are usually regarded as the indexes of drug-induced liver injury in clinics (Borlak et al., 2014). Numerous studies have shown that drugs and other chemical substances can injure hepatocytes, leading to hepatic dysfunction (Sun et al., 2014; Wang et al., 2014). However, the mechanism of F-induced liver dysfunction remains unclear. In this experiment, the biomakers related to liver functions and the abilities of liver oxidation and antioxidation were detected to explore the effects of oxidation stress on F-induced liver dysfunction in female mice.

2. Materials and methods

2.1. Experimental animals

A total of 72 3-week-old healthy female Kunming mice were obtained from the Experimental Animal Centre of Zhengzhou University, and kept in a standard animal housing at 22–25 °C, with ventilation and hygienic conditions.

2.2. F exposure

The mice were randomly divided into two groups of 36 mice. The mice in the negative control group (NC group) were reared on a standard diet and given *ad libitum* access to water. The mice in the F group were given a standard diet and drinking water containing 100 mg F/L. On the 70th day of treatment, the mice were anesthetised with 20% urethane (ethyl carbamate) solution. Blood was collected by cardiac puncture, clotted at room temperature for 30 min, and then centrifuged at 3000*g* for 10 min. Serum samples were stored at −80 °C. Livers were removed immediately, and parts of the tissue were rinsed in physiological saline (0.85% NaCl), then quickly fixed in 4% neutral formaldehyde for microscopic study and 2.5% glutaraldehyde for ultrastructural study, respectively. The experimental design was approved by the Institutional Animal Care and Use Committee of China.

2.3. Assay of the biochemical makers of oxidative stress in liver and serum

After collection, the liver tissues were homogenized in ninefold (*w*/*v*) cold normal saline using an automatic homogeniser, and then centrifuged at 2000*g* for 10 min. The supernatants were used to determine the ROS content, the activities of SOD, GSH-Px, and T-AOC and the concentrations of MDA and T-NO. Serum samples were also used to determine the biochemical makers. These biochemical makers were measured spectrophotometrically according to the standard procedures using commercially available diagnostic kits that were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

2.4. Quantitative real-time PCR of Cat, GSH-Px1, NOS2 and SOD1 mRNA expression

Total cellular RNA was extracted from the liver tissue using Trizol Reagent (Invitrogen, USA), followed by application of RNase-free DNase I to remove the contaminated DNA, quantified on a spectrophotometer (Eppendorf, Germany), and stored at −80 °C until use. Specific primers for Cat, GSH-Px1, NOS2, SOD1 and GAPDH genes were designed using Primer 5.0 software (Table 1). The relative expression levels of Cat, GSH-Px1, NOS2 and SOD1 mRNA were performed determined with a Mx3000P™

Table 1
Primer sequences with their corresponding PCR product size and position.

Gene	Primer sequence (5′–3′)	Primer locations	Product (bp)	Genbank accession no.
GAPDH	ACCCAGAAGACTGTGGATGG CACATTGGGGGTAGGAACAC	615–785	171	GU214026.1
Cat	ACATGGTCTGGGACTTCTGG CAAGTTTTTGATGCCCTGGT	626–822	197	NM62897.1
GSH-P_X1	GAGGGTAGAGGCCGGATAAG AGAAGGCATACACGGTGGAC	73–285	213	NM_008160.6
NOS2	CACCTTGGAGTTCACCCAGT ACCACTCGTACTTGGGATGC	881–1050	170	AF065922.2
SOD1	CCAGTGCAGGACCTCATTTT TTGTTTCTCATGGACCACCA	292–488	197	NM_011434.1