Chemosphere 211 (2018) 112–119

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Pre-and postnatal exposition to fluorides induce changes in rats liver morphology by impairment of antioxidant defense mechanisms and COX induction

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HIGHLIGHTS

- Exposure of rats to NaF caused an accumulation of F^- in liver, but not in serum.
- The changes may be caused by COX-2 dependent PGE2 synthesis.
- F^- caused inhibition of antioxidative enzymes activity.
- Small vesicles were apparent in liver, indicating the early phase of steatosis.

ARTICLE INFO

Article history:
Received 26 April 2018
Received in revised form
21 July 2018
Accepted 23 July 2018
Available online 26 July 2018

Handling Editor: A. Gies

Keywords:
Fluoride
Toxicity
Liver
Inflammation
Cyclooxygenase
Steatosis

ABSTRACT

Introduction: Fluorides are common in the environment and are absorbed mostly in the stomach and gut, it can easily move through cell membranes and its accumulation can cause harmful effects in skeletal and soft tissues. One of the most important F- accumulation sites is the liver.
The aim of this study was to determine whether F- can cause inflammation in rat liver by affecting the activity of antioxidant enzymes and changes in the synthesis of prostaglandin E2 (PGE2) and thromboxane B2 (TXB2).
Materials and methods: An *in vivo* model of prenatal and postnatal exposure to sodium fluoride (NaF) was used to carry out the experiment. Animals from control group received tap water to drink, while animals exposed to F- received drinking water containing NaF, 50 mg/L. In serum and liver we analyzed F- concentration, in liver - antioxidant enzymes activity, PGE2 and TXB2 concentration and immunolocalization of COX1 and COX2 proteins were measured.
Results: We observed significant changes in F- concentration only in liver. The results of this study showed that F- affects antioxidant enzymes activity, COX2 protein expression and PGE2 synthesis in liver. Also, in some regions of the liver of rats exposed to F-, the hepatocytes were diffusely altered, with changes resembling microvesicular steatosis.
Conclusion: Chronic exposure to F^- during development causes an accumulation of this element in the liver and changes in antioxidant enzymes activity and cyclooxygenase expression. Long term exposure to this element is toxic to the liver and can cause disturbances in its homeostasis.

1. Introduction

Fluorine compounds (fluorides) are very common in the environment, occurring naturally in the air, soil and ground water

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https://doi.org/10.1016/j.chemosphere.2018.07.145
0045-6535/

(Perumal et al., 2013). Dust, oral care and food products all contribute to the total daily exposure to fluoride (F^-) but the most important – due to high consumption – is drinking water. The greatest levels of contamination with F^- are reported in many areas in Africa, Asia and South America (WHO, 2006).

Fluoride is absorbed mostly in the stomach and gut. It can easily move through cell membranes and its accumulation can cause harmful effects in skeletal and soft tissues (Dec et al., 2017; Son et al., 2010). One of the most important F^- accumulation sites is the liver, receiving 70% of blood supply from the intestinal outflow. The liver is the first line of the defense against toxicants derived from the gut (Son et al., 2010). The ingestion of excessive amounts of F^- affects liver metabolism and disturbs its homeostasis (Niu et al., 2016b). Long-term exposure to this element stimulates hepatocellular necrosis and apoptosis, tissue degeneration, inflammation and carcinogenesis (Blaszczyk et al., 2011; Jha et al., 2012; Song et al., 2015). The mechanisms of F^- toxicity are not fully understood, but it has been shown that it is mainly associated with an increased production of ROS (reactive oxygen species) – particularly the superoxide anion, oxidative stress, increased lipid peroxidation and changes in the activity of many enzymes (Barbier et al., 2010; Gutowska et al., 2010; Izquierdo-Vega et al., 2008). Liver function tests, including analysis of the activity of serum enzymes such as alanine aminotransferase (ALT), alanine phosphatase (ALP) and lactate dehydrogenase (LDH), have shown that exposure to F^- leads to an increased activity of these enzymes in blood (Borlak et al., 2014). Antioxidative defense, including non-enzymatic antioxidants e.g. glutathione and specialized enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR), can also be affected by exposure to F^- (Flora et al., 2009).

Disturbances in antioxidant enzyme activity in soft tissues may cause initiation and progression of inflammation due to the activation of proinflammatory enzyme pathways, such as cyclooxygenases (COX-1 and COX-2) which are involved in arachidonic acid (AA) metabolism into prostanoids (e.g. prostaglandin E2 and thromboxane A2) which act as local hormones (Williams et al., 1999). Traditionally, COX-1 was defined as a constitutive COX isoform and had been considered to be involved only in physiological processes (Morita, 2002; Williams et al., 1999). According to a recent concept, COX-1 is also involved in inflammation and contributes to the resolution of inflammation (Gilroy and Colville-Nash, 2000; Morita, 2002). COX-2 expression is associated mainly with inflammation and can be induced by various proinflammatory stimuli including F^- (Gutowska et al., 2015; Lessner et al., 2002).

Therefore, in this study we decided to examine whether F^- can affect inflammation in rat liver by measuring the activity of antioxidant enzymes and the synthesis of COX products such as PGE2 and TXB2.

2. Material and methods

2.1. Animal model

An *in vivo* model of prenatal and postnatal exposure to sodium fluoride (NaF) was used to carry out the experiment. Procedures involving animals were carried out in strict accordance with international standards of animal care guidelines and every effort was made to minimize suffering and the number of animals used. Experiments were approved by the Local Ethical Committee on Animal Testing in Szczecin, Poland (approval No 32/2015). Adult female Wistar rats (3 months old; $n = 6$) were kept in a cage with sexually mature males (2:1) for one week. All animals had free access to food (standard diet) and drinking water *ad libitum*. The cages stood in a room at a controlled temperature under a 12 h/12 h light/dark regime. After a week the females were separated from the males and each female was placed in an individual cage. Pregnant females were randomly divided into two groups. Animals from group I (Control, $n = 3$), received tap water to drink, while animals from experimental group II (Fluoride, $n = 3$) received drinking water containing NaF, 50 mg/L. The volume of intaken liquids did not differ significantly between the experimental and control rats. The experiment lasted from pregnancy day 0 to postnatal day 90 (PND 90). During the experiment pups were separated from the mothers and placed in new cages at PND 21 - after the end of breast-feeding. The young rats were kept in the same conditions as previously until the end of the experiment.

We chose oral administration of sodium fluoride as it reflects environmental exposure. Exposure of rats to F^- in drinking water with NaF at 50 mg/L is based on commonly used models in the analyses of F^- toxicity, which show that a 5 times higher concentration of this element should be used in rats to achieve an effect similar to that observed in the human body. According to this model, the dose of 50 mg/L in rats is equivalent to human exposure to the concentration of F^- in drinking water at 10 mg/L (Pereira et al., 2016). It should be noted, however, that rats consume about 30 mL–50 mL of water per day, which when given 50 mg/L gives an intake of about 1.5 mg–2.5 mg of F^- with water throughout the day. The norms of consumption of this element according to the Polish standard SAI (Safe and Adequate Daily Intake) and ADI (Acceptable Daily Intake) amount to 3 mg - 4 mg/day for an adult (depending on gender), and retrospective studies have shown that the symptoms of fluorosis in an adult human weighing 70 kg appear with a consumption of more than 10 mg of F^- per day (Flores-Mendez et al., 2014).

After the exposure, PND 90, animals were sacrificed by decapitation, livers were dissected and placed in liquid nitrogen. Samples were stored at −80 °C for later analysis.

2.2. The measurement of fluoride concentration in rats serum and liver

2.2.1. The measurement of fluoride concentration in rats serum

To measure the concentration of fluoride in the serum, 0.5 mL of 5% TISAB III buffer solution was added to 0.5 mL of the serum portion. The potentiometry method and the ionselective electrode were used.

2.2.2. The measurement of fluoride concentration in rats liver

To measure the concentration of fluoride in liver, the tissue of animals from control and study group has been dry in 95 °C to receive dry-matter. Then the samples were homogenized in ceramic mortars and 10 mg test portions of samples were prepared from each one. Those samples portions were mineralized for 1 h in 95 °C with 1 mL of perchloric acid ($HClO_4$). Subsequently 0.5 mL of obtained solution, 2,5 mL of TISAB buffer and 2 mL of 1 M sodium citrate were transferred into polyethylene cups and then the fluoride concentration was measured using ionselective electrode.

2.3. The measurement of antioxidant enzymes activity and glutathione concentration in liver

2.3.1. Superoxide dismutase (SOD)

SOD activity was measured using the Superoxide Dismutase Assay Kit (Cayman Chemical Company, USA) and ASYS UVM 340 (Biogenet) spectrophotometer. SOD is a metalloenzyme that catalyze the dismutation of the superoxide anion to molecular oxygen and hydrogen peroxide and thus form a crucial part of the cellular antioxidant defense mechanism. In this method tetrazolium salt is utilized by SOD for detection of superoxide radicals generated by xanthine oxidase and hypoxanthine (Cayman Chemical, 2016a).