Journal of Fluorine Chemistry 168 (2014) 212–217

Contents lists available at ScienceDirect

Journal of Fluorine Chemistry

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

Effects of chronic fluoride intake on the antioxidant systems of the liver and kidney in rats

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ARTICLE INFO

Article history:
Received 22 August 2014
Received in revised form 25 September 2014
Accepted 27 September 2014
Available online 6 October 2014

Keywords:
Fluoride
Antioxidants
Oxidative stress
Liver
Kidney

ABSTRACT

Excessive fluoride intake over a long period of time can lead to fluorosis, which may cause dental and skeletal manifestations. Metabolic, functional and structural damage caused by chronic fluorosis have been reported in many tissues, but the exact mechanisms modulated by fluoride remain unclear. The aim of this study was to evaluate the effect of fluoride administered in drinking water on the antioxidant defense system of rats. Four groups of *Wistar* rats were used for the study ($n = 10$/group). The animals received drinking water containing 0 (control), 5, 15 or 50 mg/L of fluoride over 60 days. They were then euthanized, and their livers and kidneys were collected and homogenized. Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH), antioxidants, thiobarbituric acid reactive substances (TBARS), lipid hydroperoxide (LH), and fluoride levels were analyzed. Data were analyzed by ANOVA and Tukey's test or by the Kruskal–Wallis and Dunn's tests ($p < 0.05$). In the kidneys, the SOD, GPx, GSH and antioxidants levels significantly decreased, while the fluoride and LH levels significantly increased. In the liver, the CAT and TBARS levels significantly decreased, while the fluoride, SOD, LH and antioxidants levels significantly increased. In summary, these results show that chronic fluoride administration alters the antioxidant system of rats. Our data suggest that the conversion of the superoxide anion to water in the kidney upon exposure to high levels of fluoride occurs mainly through SOD and CAT and not through the glutathione system, in contrast to what occurs in the liver.

1. Introduction

Excessive uptake of fluoride can cause fluorosis, a condition characterized by an altered appearance of the teeth, such as pitting or staining of the enamel, and skeletal manifestations at higher doses, such as bone deformities, osteoporosis and osteosclerosis. Endemic fluorosis is widely prevalent around the world and affects millions of people [1].

Fluoride can affect cells in several ways, depending on the time of exposure, concentration and cell type. At micromolar levels, fluoride is an anabolic agent and promotes cell proliferation. However, millimolar concentrations inhibit several enzymes, including phosphatases, both *in vivo* and *in vitro* [2]. Stimulation of enzyme activity by fluoride has also been reported [3].

Metabolic, structural and functional damage as a result of chronic fluorosis have been identified in different tissues, including renal, endothelial, gonadal and neuronal cells [4]. Oxidative stress following excessive fluoride exposure has been observed in several cell types *in vitro* and in soft tissues *in vivo* [5–7]. Fluoride affects the liver, spleen, brain, lungs and testicles of animals and humans living in endemic areas of fluorosis. Fluoride is reported to inhibit the activity of antioxidant enzymes such as SOD, GPx and CAT [5,6,8,9]. In addition, fluoride can alter glutathione levels [9–12], causing excessive production of ROS at the mitochondrial level and damaging some cell components. In addition, fluoride can cause neuronal damage in rats treated with 600 ppm for one week due to increased oxidative stress in the brain [13].

While the effects of fluoride on the antioxidant system have been evaluated in many studies, the methodologies vary greatly,

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http://dx.doi.org/10.1016/j.jfluchem.2014.09.029
0022-1139/

which impairs comparisons of the results obtained in different tissues. Furthermore, the doses of fluoride used in many cases are extremely high and do not have clinical significance [13,14]. The aim of this study was to evaluate the effects of clinically significant doses of fluoride on the antioxidant systems of the liver and kidneys of rats.

2. Results and discussion

There were no significant differences in either the mean body weight or kidney weight between the groups (results not shown), which is consistent with the literature for the fluoride doses used [15]. The mean liver weight was higher in the experimental groups compared to the control group ($p = 0.0064$). However, only the 15 mg/L fluoride group (8.43 ± 0.57 g) significantly differed from control (7.33 ± 0.85 g); the increases observed in the 5 mg/L (8.03 ± 0.55 g) and 50 mg/L fluoride groups (7.90 ± 0.37 g) were not significant. However, when the ratio liver weight/body weight was considered, both the 5 and 15 mg/L fluoride groups presented ratios significantly higher than the one observed for the control group. It has been reported that exposure to higher fluoride doses (such as 100 mg/L) reduces the weight of the liver of rats [16].

After intake, fluoride is absorbed in the gastro-intestinal tract and reaches systemic circulation [17]. Blood plasma is an important indicator of fluoride intake levels in cases of both acute [18–20] and chronic exposure [15,18,21–23]. An increase in plasma fluoride levels was observed as water fluoride concentrations were increased in the present study, which is consistent with the literature [15,22,23] (Table 1). Fluoride is distributed to the whole organism *via* plasma and is excreted in the urine [17]. In the present study, the group treated with 5 mg/L fluoride displayed plasma fluoride levels similar to those of the control group, consistent with previous studies using the same treatment protocol [15,21–23], and could be partially explained by fluoride uptake in mineralized tissues [17].

Fluoride concentrations in the liver and kidney are shown in Table 1. In the kidney, a dose-dependent response was observed, with significant differences between all groups ($p < 0.0001$). Significant differences in the liver fluoride concentrations were also observed among the groups ($p < 0.0001$). The 50 mg/L fluoride group displayed the highest fluoride concentration, and both the 15 mg/L and 50 mg/L groups had significantly increased fluoride levels compared to the control group, which displayed the lowest concentration. The kidneys are the main organs responsible for the reduction of plasma fluoride levels after fluoride intake; approximately 60% of the fluoride absorbed is excreted in the urine in healthy adults [24]. As the kidneys are the principal route for fluoride elimination from the organism, the fluoride levels in the kidneys displayed a dose-dependent effect in the kidneys in the present study: the fluoride levels increased upon exposure to higher concentrations of fluoride. In contrast, the fluoride levels in the liver (Table 1) decreased compared to those found in the plasma. This result was expected because intracellular fluoride levels are typically 10–50% lower than those found in plasma or interstitial fluid [17].

Table 1
Fluoride Concentration in the plasma (μg/mL) and the liver and kidney (μg/g tissue) of rats receiving water containing different fluoride concentrations.

	Control	5 mg/L	15 mg/L	50 mg/L
Plasma	0.032 ± 0.011^{a}	0.027 ± 0.008^{a}	0.043 ± 0.010^{a}	0.094 ± 0.019^{b}
Liver	0.009 ± 0.003^{a}	0.010 ± 0.002^{ad}	0.014 ± 0.004^{bd}	0.023 ± 0.008^{c}
Kidney	0.045 ± 0.012^{a}	0.067 ± 0.011^{b}	0.112 ± 0.020^{c}	0.245 ± 0.042^{d}

Values are mean ± SD ($n = 10$). In each line, different superscript letters indicate significant differences among the groups ($p < 0.05$).

Macroscopic analysis of the liver did not reveal any differences between the groups, and all groups displayed normal morphological characteristics, such as uniform appearance, intact hepatic lobules, intact portal area, defined hepatic vein, and intact sinusoids converging to the central lobule vein. However, the group treated with 50 mg/L fluoride (Fig. 1B) displayed more disorganized hepatocytes compared to the control group (Fig. 1A), and some hepatocytes were observed to have heterogeneous nuclear sizes, disorganized architecture and changes in the delimitation of the cytoplasm. Signs of toxicity include changes in organ weights and hematological and biochemical blood alterations [25]. We did not observe either macroscopic alterations or microscopic signs of toxicity. However, the group treated with 50 mg/L of fluoride displayed areas with altered hepatocytes; similar results were described by Bouaziz et al. [26], who observed liver changes in mice intoxicated by fluoride.

Histological analysis of the kidneys did not reveal any differences between the groups. Discrete vascular congestion was observed only in the group treated with 50 mg/L of fluoride (Fig. 1C). This group also displayed a higher number of congested vessels compared to the control group, but over less than 25% of the tissue (Fig. 1D). Vascular congestion has also been described by other authors. Kobayashi et al. [22] also observed vascular congestion, but found no classic signs of nephropathy induced by fluoride. Hyperemia (vascular congestion) can be an early sign of the inflammatory response [27]. Other studies on the chronic toxicity of fluoride have also reported the presence of vascular congestion [8,22,28].

CAT activity in the kidneys (Table 2) was similar in all groups, and no significant differences were observed ($p = 0.4157$). However, CAT activity in the liver (Table 3) was significantly different among the groups ($p = 0.0001$). All the experimental groups displayed decreased CAT activity compared to the control group; the 15 mg/L fluoride group displayed the lowest value and was significantly different from both the control and the 5 mg/L fluoride groups.

The SOD activity in the kidney was also significantly different between the groups ($p = 0.0012$; Table 2). The 50 mg/L fluoride group displayed the lowest value (approximately a 50% decrease) and significantly differed from both the control and the 5 mg/L fluoride groups. No significant differences were observed between the control group and the 5 mg/L and 15 mg/L fluoride groups. SOD activity in the liver was also significantly different between the groups ($p = 0.0006$): the 15 mg/L fluoride group displayed the highest activity and significantly differed from both the control and 5 mg/L fluoride groups (Table 3).

The GPx activity (Table 2) in the kidney was significantly different between the groups ($p < 0.0001$). The 50 mg/L fluoride group displayed the lowest activity, while the 5 mg/L group displayed the highest activity. In the liver, the GPx activity increased in all the experimental groups compared to the control group, but no significant differences were detected ($p = 0.4964$) (Table 3).

The GSH levels in the kidney (Table 2) were significantly different between the groups ($p = 0.0177$). The concentration decreased in all experimental groups compared to the control group, but the decrease was only significant for the 50 mg/L fluoride group. In the liver (Table 3), all experimental groups displayed increased GSH concentrations, but no significant differences were detected ($p = 0.2996$).

The LH levels in the kidney and liver are shown in Tables 2 and 3, respectively. The LH levels in the kidney were significantly different between the groups ($p = 0.0075$). All experimental groups displayed increased concentrations compared to the control group, but only the 15 mg/L fluoride group significantly differed from the control. The LH levels in the liver were also significantly different