OXIDATIVE STRESS FROM FLUORIDE-INDUCED HEPATOTOXICITY IN RATS

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SUMMARY: The role of oxidative stress in fluoride hepatotoxicity was investigated. Three groups of eight 4-week-old Wistar rats were given 50, 100, and 150 mg NaF/L in their drinking water for three months. Serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxalate transaminase (SGOT) activities increased significantly, suggesting hepatic damage. Alterations of the oxidative and the antioxidant system in the liver were confirmed by the significant increase in malondialdehyde (MDA) together with enhanced superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities in that organ. Oxidative damage from oxidative stress therefore appears to be an important pathway for fluoride-induced hepatotoxicity.

Keywords: Fluorosis; Hepatotoxicity; Liver transaminases; Oxidative stress; Rat liver study.

INTRODUCTION

Endemic fluorosis is caused by excessive fluoride ingestion over a prolonged period. In addition to well-known effects on the skeleton and teeth, fluorosis can adversely affect many tissues and organs as exhibited by a broad array of symptoms and pathological changes.[1] Endemic fluorosis is thus a severe hazard to human health and often a serious health problem in a number of developing countries. However, the manner in which the whole-body effects are produced is still unclear, and efforts to prevent and treat fluorosis by therapeutic measures have had only limited success.

Epidemiological investigations and animal experiments indicate that the histological structure and function of liver are altered in animals and humans with fluorosis,[1-3] but the mechanisms are not fully understood. With further investigations of the involvement of free radicals, increased generation of oxygen radicals and enhanced lipid peroxidation have been shown to play an important role in fluorosis.[4,5,13,14]

To examine oxidative stress in hepatic damage caused by fluoride, the effect of sodium fluoride ingestion on the activities of serum glutamate pyruvate transaminase (SGPT) and glutamate oxalate transaminase (SGOT) were studied in an animal experiment, together with the content of malondialdehyde (MDA) and the activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) in the liver.

MATERIALS AND METHODS

Thirty-two healthy 4-week-old Wistar rats, weighing between 90-100 g at the beginning of the study, were obtained from the Laboratory Animal Center of China Medical University. The rats were randomly divided into four

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groups of 8 animals (4 males and 4 females, housed in two separate stainless steel cages in each group). During the study, all rats were on a 12-hr light-dark cycle in a room with the relative humidity ranging from 30% to 55% and the temperature between 22º C and 25º C. The animals in the control group were given distilled water to drink. The other three groups were given drinking water containing 50 mg/L, 100 mg/L and 150 mg/L of sodium fluoride (NaF), respectively. All the animals were maintained on standard chow, and water was available *ad libitum*.

After three months the rats were sacrificed under light ether anesthesia. For the serum assays, blood was collected by cardiac puncture. For the hepatic assays, the liver was dissected out carefully, blotted free of blood, weighed, homogenized, and centrifuged. The supernatant was then assayed for the activities of SOD,[6] GSH-Px,[7] and the content of MDA.[8] The protein content of the homogenates was determined by the method of Bradford.[9] Fluoride in urine and serum was measured with a CSB-F-I fluoride ion electrode.[10]

Data are expressed as means ± SEM. The significance of the difference between means was determined by analysis of variance (ANOVA). A value of $p<0.05$ was considered significant.

RESULTS

Fluoride content in urine and serum: The fluoride content in urine and serum in the three experimental groups was significantly higher than in the control group ($p<0.01$) in a dose-dependent manner (Table 1).

Table 1. Fluoride content in urine and serum of rats after 3 months

NaF (mg/L) (n=8 per group)	Urine (mg/L)	Serum (mg/L)
0	0.57 ± 0.18	0.03 ± 0.007
50	3.21 ± 1.54*	0.09 ± 0.03†
100	8.48 ± 1.31†	0.11 ± 0.03†
150	12.96 ± 3.77†	0.13 ± 0.02†

Compared with the control group, *$p<0.05$; †$p<0.01$.

Activities of SGPT and SGOT in serum: The activities of SGPT and SGOT in the serum were elevated in a dose-dependent manner following NaF treatment. The activity of SGPT in the 150 mg NaF/L group was significantly higher than in the control group ($p<0.01$). The activities of SGOT in the 100 mg NaF/L and the 150 mg NaF/L groups were both significantly higher than in the control group ($p<0.01$) (Table 2).

Table 2. Activities of SGPT and SGOT in serum of rats after 3 months

NaF (mg/L) (n=8 per group)	SGPT (U/L)	SGOT (U/L)
0	12.50 ± 2.07	32.25 ± 8.76
50	15.00 ± 4.73	40.00 ± 5.69
100	16.90 ± 2.59	46.13 ± 5.89*
150	24.80 ± 11.66*	46.88 ± 8.34*

Compared with the control group, *p<0.01.

Oxidative stress in hepatic tissue: The content of MDA in the 150 mg NaF/L group increased significantly compared with the control group. There was a positive correlation between MDA and the fluoride content in serum ($r = 0.559$; $p<0.01$). The activity of SOD decreased with the increased concentration of fluoride. The activities of SOD in the 100 mg NaF/L and the 150 mg NaF/L groups decreased significantly compared with the control group ($p<0.05$). There was a negative correlation between the activity of SOD and the fluoride content in serum ($r = –0.563$; $p<0.01$). The activity of GSH-Px decreased with increased concentration of fluoride, but there were no significant differences among the four groups (Table 3).

Table 3. Content of MDA and the activities of GSH-Px and SOD in hepatic tissues of rats after 3 months

NaF (mg/L) (n=8 per group)	MDA (nmol/mg.pr)	SOD (NU/mg.pr)	GSH-Px (U/mg.pr)
0	0.63 ± 0.22	116.55 ± 19.58	8.44 ± 5.33
50	1.40 ± 0.82	110.71 ± 20.52	7.33 ± 3.29
100	2.72 ± 3.34	99.62 ± 10.30*	5.93 ± 5.80
150	5.12 ± 3.25†	96.92 ± 8.40†	4.58 ± 3.65

Compared with the control group, *p<0.05; †p<0.01.

DISCUSSION

Excessive fluoride exposure for a prolonged period can induce chronic fluorosis. In this study, after three months with the various indicated concentrations of fluoride in their drinking water, the rats had fluoride levels in their urine and serum that were significantly higher than those of the control group. Dental fluorosis was also observed in the rats of the experimental groups.

Reactive oxygen species (ROS) are implicated as important pathological mediators in many types of disorders. Their presence is reflected by increased oxygen radical generation and lipid peroxidation in the pathogenesis of many diseases as well as the toxic action of a wide range of compounds.[11,12] Numerous studies have examined the relationship between fluoride and free radical reactions.[4,5,13,14] Some investigations indicate that excessive fluoride can induce lipid peroxidation, which involves polyunsaturated fatty acids.[15] Reduced activities of antioxidant enzymes and lowered contents of antioxidants were also found in kidney and liver of animals treated with fluoride, which may result in heavy accumulation of free radicals.[13,14,16] All these results indicate that free radicals play an important role in the pathogenesis of fluorosis.

Liver is an important organ for metabolism and detoxification. SGPT and SGOT are markers of liver function. Drinking high-fluoride water over a long period can damage the liver. In the present study, elevated activities of SGPT and SGOT demonstrated liver damaged in the fluorotic rats, in agreement with results of previous investigations.[1-3]

Under normal conditions, free radical production and elimination are in a dynamic balance. Oxidative stress produced by free radicals and hydrogen peroxide is greater if fluoride impairs the production of free radical scavengers such as GSH, GSH-Px, SOD, and ascorbic acid. In the present study the content of MDA increased and the activities of antioxidant enzymes decreased in the liver of fluoride-treated rats. The results suggest that the balance between the oxidative system and antioxidant system in the rats was broken during fluoride exposure. Liver contains considerable amounts of polyunsaturated fatty acids, which are prone to damage by free radicals. Our results suggest that oxidative stress played an important role in the hepatic damage induced by fluoride.

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