

## EFFECTS OF FLUOROSIS ON LIPID PEROXIDATION AND ANTIOXIDANT SYSTEMS IN YOUNG PIGS

Xiu-an Zhan,<sup>a</sup> Zi-rong Xu,<sup>a</sup> Jian-xin Li,<sup>a,b</sup> Min Wang<sup>a</sup>

Hangzhou, China

**SUMMARY:** This study was conducted to investigate effects of fluoride on lipid peroxidation and antioxidant systems in young pigs. Three groups of crossbred barrows about 50 days old were exposed to 100, 250, and 400 mg F<sup>-</sup>/kg (from NaF) in their diets for 50 days. Serum malondialdehyde (MDA) and nitric oxide (NO) levels were significantly increased, and the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) were significantly decreased. In thyroid, liver, and kidney tissues, the MDA level was significantly increased, and SOD and GSH-Px activities were significantly decreased. These results suggest that fluoride induces excessive production of NO and reactive oxygen species (ROS), enhances lipid peroxidation, and disturbs the antioxidant system of pigs. Oxidative damage from oxidative stress could therefore be an important pathway for fluoride toxicity in soft tissues.

Keywords: Catalase; Fluoride; Glutathione peroxidase; Malondialdehyde; Nitric oxide; Oxidative stress; Reactive oxygen species; Superoxide dismutase; Young pigs.

### INTRODUCTION

Excessive intake of fluoride for a prolonged period can induce chronic fluorosis. Because mineral supplements such as calcium phosphate and limestone often contain high levels of fluorine, chronic fluorosis of animals can result with great economic loss in animal production.

Fluoride produces deleterious effects on the skeleton, teeth, and soft tissues,<sup>1-8</sup> but the mechanisms are not fully understood. In recent decades, numerous investigations have focused on the relationship between fluoride and free radical reactions. Many studies indicate that excessive fluoride can induce free radical toxicity in humans and animals.<sup>5,6,9-12</sup>

The present study was undertaken to assess effects of fluoride on lipid peroxidation and antioxidant systems in young pigs to obtain information for effective prevention and treatment of fluorosis.

### MATERIALS AND METHODS

Thirty-two (about 50-day-old) barrows (Duroc x Landrace x Yorkshire) with an average body weight of about 17 kg, obtained from Zhenning Animal Husbandry Co., Ltd (Ningbo, China), were allowed to acclimatize to the study conditions for one week and then allotted randomly to four groups of eight each. They all received the same basal diet based on corn-soybean meal containing 6.2 mg F<sup>-</sup>/kg. Group 1 was designed as the control, and the other three groups, designated as groups 2, 3, and 4, were fed the same basal diet but supplemented with 100, 250, and 400 mg F<sup>-</sup>/kg (from NaF), respectively. Diets were formulated to meet

---

<sup>a</sup>Feed Science Institute, College of Animal Science, Zhejiang University, No.268 Kaixuan Road, Hangzhou, China, 310029.

<sup>b</sup>For Correspondence: Jian-xin Li. E-mail:ljx536@yahoo.com.cn

or exceed nutrient requirements suggested by the NRC (1998).<sup>13</sup> All groups had *ad libitum* access to feed and to tap water containing 0.6 mg F<sup>-</sup>/L.

On the 50th day of the feeding trial, all pigs were deprived of feed for 12 hr and then slaughtered under general anaesthesia. For serum assays, blood samples were collected by cardiac puncture. For the hepatic, nephritic, and thyroid assays, the liver, kidney, and thyroid were carefully dissected out and blotted free of blood. These samples were immediately quick-frozen in liquid nitrogen and stored at -70 °C prior to analysis.

Serum malondialdehyde (MDA)<sup>14</sup> and nitric oxide (NO)<sup>15</sup> levels along with superoxide dismutase (SOD),<sup>16</sup> glutathione peroxidase (GSH-Px),<sup>17</sup> and catalase (CAT)<sup>18</sup> activities were determined by the methods cited. Liver, kidney, and thyroid tissues were homogenized, and the tissue homogenate level of MDA<sup>14</sup> and the activities of SOD<sup>16</sup> and GSH-Px<sup>17</sup> were assayed. The protein content of the homogenates was determined by the method of Bradford,<sup>19</sup> and the SOD and GSH-Px activities were expressed in terms of units/mg protein.

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

## RESULTS

In serum, the MDA level was significantly higher, and the activities of SOD and GSH-Px were significantly lower in all fluoride-treated groups than in the control group. There was also a significant increase in the NO level and a decrease in CAT activity in groups 3 and 4 (Table 1).

**Table 1.** MDA, NO, and antioxidants in serum of young pigs

Group <sup>a</sup>	1	2	3	4
MDA (nmol/mL)	5.97±1.50	8.21±2.91*	8.19±1.68*	9.36±1.09 <sup>†</sup>
NO (µmol/L)	27.22±11.71	31.94±20.37	69.42±31.52 <sup>†</sup>	51.06±18.34*
SOD (U/mL)	75.80±6.71	65.99±5.52*	62.98±8.97 <sup>†</sup>	63.11±5.45 <sup>†</sup>
GSH-Px (U/mL)	802.72±63.38	650.04±106.69 <sup>†</sup>	639.80±99.12 <sup>†</sup>	613.03±153.39 <sup>†</sup>
CAT (U/mL)	6.88±1.59	5.99±1.55	3.80±1.91 <sup>†</sup>	4.86±1.93*

Values are mean±SD. Compared with the control group, \* $p < 0.05$ ; <sup>†</sup> $p < 0.01$ .

<sup>a</sup>Group 1 was the control group. Groups 2, 3 and 4 were the experimental groups.

In the thyroid and liver homogenates, the MDA level was significantly elevated, whereas the SOD activity was significantly lower in groups 3 and 4. GSH-Px activity was also significantly decreased in all fluoride-treated groups compared with the control group. In groups 3 and 4 the kidney homogenates had a significantly elevated MDA level and a lowered GSH-Px activity. The SOD activity was significantly decreased in all fluoride-treated groups (Table 2).

**Table 2.** MDA and antioxidants in thyroid, liver, and kidney homogenates of young pigs

Group <sup>a</sup>	1	2	3	4
MDA (nmol/mg protein)				
Thyroid	0.15±0.05	0.20±0.05	0.26±0.04 <sup>†</sup>	0.26±0.10 <sup>†</sup>
Liver	0.34±0.11	0.44±0.16	1.21±0.41 <sup>†</sup>	1.12±0.35 <sup>†</sup>
Kidney	0.46±0.09	0.54±0.09	0.63±0.16*	0.72±0.20 <sup>†</sup>
SOD (units/mg protein)				
Thyroid	66.80±8.03	57.21±13.34	50.80±10.08 <sup>†</sup>	45.67±7.40 <sup>†</sup>
Liver	235.13±30.30	226.55±54.59	172.43±33.97 <sup>†</sup>	180.96±54.74*
Kidney	136.58±19.82	116.77±19.46*	110.50±13.60 <sup>†</sup>	111.87±18.24 <sup>†</sup>
GSH-Px (units/mg protein)				
Thyroid	1.91±0.23	1.65±0.12*	1.53±0.23 <sup>†</sup>	1.43±0.32 <sup>†</sup>
Liver	15.18±2.50	11.13±1.61 <sup>†</sup>	9.79±2.82 <sup>†</sup>	8.69±1.93 <sup>†</sup>
Kidney	13.04±2.68	10.59±2.05	8.59±1.82 <sup>†</sup>	9.75±3.68 <sup>†</sup>

Values are mean±SD. Compared with the control group, \*p<0.05; <sup>†</sup>p<0.01.

<sup>a</sup>Group 1 was the control group, groups 2, 3 and 4 were the experimental groups.

## DISCUSSION

During rearing and slaughter, lassitude, anorexia, and sluggishness were observed in the fluoride-treated pigs. Pathological lesions were also observed in thyroid, liver, and kidney tissues, in agreement with previous investigations.<sup>8,20,21</sup>

Reactive oxygen species (ROS) are implicated as important pathological mediators in many types of disorders. Increased oxygen radical generation and enhanced lipid peroxidation are associated with the pathogenesis of many diseases and the toxic action of a wide range of compounds.<sup>22,23</sup> Many investigations indicate that excessive fluoride can enhance lipid peroxidation and inhibit the antioxidative enzymes in liver, kidney, heart, ovary, brain, and gastrocnemius muscle of animals treated with fluoride.<sup>5,6,9-12</sup> The present study also indicated that fluoride increased free radical production and inhibited the antioxidative enzymes in the thyroid, liver, and kidney, which probably made the tissues more susceptible to biochemical injury.

But there are some contrary reports. Reddy et al. reported finding no changes in lipid peroxides, GSH, and vitamin C levels, as well as in SOD, GSH-Px, and CAT activities in red blood cells of fluorotic humans and rabbits.<sup>24</sup> Chlubek et al.

found a 50% decrease in cytoplasmic SOD (Cu, Zn-SOD) activity and a tendency to lower mitochondrial SOD (Mn-SOD) activity in the pancreas of rats receiving 50 and 100 mg F<sup>-</sup>/L in their drinking water for four months, but no changes were noted in GSH-Px activity and MDA content.<sup>25</sup> These differences might be due to variations in animal species, ages of animals and humans exposed to fluoride, dose and mode of fluoride exposure, length of exposure, and sensitivity of different tissues to fluoride.<sup>26</sup> Meanwhile, in our study the activities of some antioxidants in the highest fluoride group 4 (serum CAT and kidney GSH-Px) were substantially increased compared to the downward trend for groups 2 and 3, although they were still significantly lower than those in the control. Such anomalies are examples of paradoxical concentration effects of fluoride,<sup>27</sup> which are often difficult to explain.

Nitric oxide (NO) is one of the reactive nitrogen species (RNS) produced in the metabolism of L-arginine to citrulline catalyzed by nitric oxide synthase. A significant increase in serum NO level was observed in the present study, in accordance with an earlier report.<sup>28</sup> NO is known for its direct and indirect toxic effects on cells. The elevated NO level induced by fluoride in an organism could disturb protein functions, influence energy metabolism, deplete ATP and NAD(P)(H), and damage DNA.<sup>29,30</sup> In addition, a more important reaction of excessive NO is combination with superoxide anions ( $\cdot\text{O}_2^-$ ), producing peroxynitrite ( $\text{ONOO}^-$ ) with stronger potential for oxidation and destruction, which is responsible for much of the cytotoxicity of NO. This anion has a fairly short half-life but is able to diffuse across cell membranes. Based on the local pH and the presence of iron, thiols, and SOD, peroxynitrite undergoes three types of reactions, leading to depletion of thiols, radical chain peroxidation, and nitrosylation of proteins.<sup>31</sup>

In conclusion, chronic fluorosis was found to cause excessive production of NO and oxygen free radicals, enhance lipid peroxidation, and inhibit the antioxidative enzymes in growing pigs. Oxidative stress induced by fluoride thus plays an important role in the pathogenesis of fluorosis.

#### ACKNOWLEDGEMENT

This research was supported by the National Basic Research Program of China (Project 2004CB117505).

#### REFERENCES

- 1 Waldbott GL. Nonskeletal fluorosis [editorial review]. *Fluoride* 1978;11:111-4.
- 2 Foulkes RG. Thirty-five years of fluoride [guest editorial]. *Fluoride* 2002;35:213-27.
- 3 Trabelsi M, Guermazi F, Zeghal N. Effect of fluoride on thyroid function and cerebellar development in mice. *Fluoride* 2001;34:165-73.
- 4 Singh M. Biochemical and cytochemical alterations in liver and kidney following experimental fluorosis. *Fluoride* 1984;17:81-93.
- 5 Guo XY, Sun GF, Sun YC. Oxidative stress from fluoride-induced hepatotoxicity in rats. *Fluoride* 2003;36:25-9.
- 6 Vani ML, Reddy KP. Effects of fluoride accumulation on some enzymes of brain and gastrocnemius muscle of mice. *Fluoride* 2000;33:17-26.

- 7 Mullenix PJ, Denbesten PK, Schunior A, Kernan WJ. Neurotoxicity of sodium fluoride in rats. *Neurotoxicol Teratol* 1995;17:169-77.
- 8 Shashi A, Singh JP, Thapar SP. Toxic effects of fluoride on rabbit kidney. *Fluoride* 2002;35:38-50.
- 9 Sharma A, Chinoy NJ. Role of free radicals in fluoride-induced toxicity in liver and kidney of mice and its reversal. *Fluoride* 1998;31:S26.
- 10 Patel PD, Chinoy NJ. Influence of fluoride on biological free radical reactions in ovary of mice and its reversal. *Fluoride* 1998;31:S27.
- 11 Sun G, Qiu L, Ding G, Qian C, Zhang Q. Effects of  $\beta$ -carotene and SOD on lipid peroxidation induced by fluoride: an experimental study. *Fluoride* 1998;31:S29.
- 12 Chinoy NJ, Patel TN. The influence of fluoride and/or aluminum on free radical toxicity in the brain of female mice and beneficial effects of some antidotes. *Fluoride* 2000;33:S8.
- 13 National Research Council. Nutrient requirements of swine. 10<sup>th</sup> revised edition. Washington (DC): National Academy Press; 1998.
- 14 Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979;95:351-8.
- 15 Stuehr DJ, Nathan CF. Nitric oxide. A macrophage product responsible for cytostasis and respiratory inhibition in tumor target cells. *J Exp Med* 1989; 169:1543 – 55.
- 16 Ji J, Wu Z, Liu Q, Zhang Y, Ye M, Li M. An ultramicroanalytic and rapid method for determination of superoxide dismutase activity. *J Nanjing Rail Med Coll* 1991;10(1):27-30 [in Chinese].
- 17 Xia Y, Zhu L. The determination of glutathione peroxidase in blood and tissue-DTNB direct method. *J Hyg Res* 1987;16(4):29-33 [in Chinese].
- 18 Beers RF, Sizer IW. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *J Bio Chem* 1952;195:133-40.
- 19 Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976;72:248-54.
- 20 Liu G, Chai C, Kang S. Effects of fluoride on thyroid structure in chicks. *Chin J Endem* 2001;20(2):90-3 [in Chinese].
- 21 Kolodziejczyk L, Put A, Grzela P. Liver morphology and histochemistry in rats resulting from ingestion of sodium selenite and sodium fluoride. *Fluoride* 2000;33:6-16.
- 22 Halliwell B, Gutteridge JMC. Oxygen free radicals and iron in relation to biology and medicine: some problems and concepts. *Arch Biochem Biophys* 1986;246:501-14.
- 23 Tappel AL. Lipid peroxidation damage to cell components. *Fed Proc* 1973; 32:1870-4.
- 24 Reddy BG, Khandare AL, Reddy PY, Rao GS, Balakrishna N, Srivalli I. Antioxidant defense system and lipid peroxidation in patients with skeletal fluorosis and in fluoride-intoxicated rabbits. *Toxicol Sci* 2003;72:363-8.
- 25 Chlubek D, Grucka-Mameczar E, Birkner E, Polaniak R, Stawiarska-Pieta B, Duliban H. Activity of pancreatic antioxidative enzymes and malondialdehyde concentrations in rats with hyperglycemia caused by fluoride intoxication. *J Trace Elem Med Biol* 2003;17:57-60.
- 26 Chlubek D. Fluoride and oxidative stress. *Fluoride* 2003;36:217-28.
- 27 Burgstahler AW. Editorial review: Paradoxical dose-response effects of fluoride. *Fluoride* 2002;35:143-7.
- 28 Liu G, Chai C, Cui L. Fluoride causing abnormally elevated serum nitric oxide levels in chicks. *Environ Toxicol Pharmacol* 2003;13:199-204.
- 29 Gross SS, Wolin MS. Nitric oxide: pathophysiological mechanisms. *Annu Rev Physiol* 1995;57:737-69.
- 30 Luperchio S, Tamir S, Tannenbaum SR. NO-induced oxidative stress and glutathione metabolism in rodent and human cells. *Free Radic Biol Med* 1996;21:513-9.
- 31 Fredstrom S. Nitric oxide, oxidative stress, and dietary antioxidants. *Nutrition* 2002;18:537-9.