Antagonistic Mechanisms of Zinc on Male Reproductive Toxicity Induced by Excessive Fluorine in Rats

Yan Li, Chunxia Jiang, Xuemin Cheng, Liuxin Cui

Department of Environmental Health, College of Public Health, Zhengzhou University, Zhengzhou, Henan 450052, China

Abstract: Objective. To explore the antagonistic mechanism of zinc on male reproductive toxicity induced by excessive fluorine. **Methods.** A total of 30 male Wistar rats were randomly allocated into three groups: control group, fluorine group, and fluorine plus zinc group. Then the animals of three groups were fed deionized water supplemented with NaF or/and $ZnSO_4$ for 6 weeks. The levels of serum testosterone (T), superoxide dismutase (CuZn-SOD), lactate dehydrogenase (LDH), and Fas expression in spermatogenic cells were detected. **Results.** The levels of T in fluorine plus zinc group significantly increased compared to control group, and fluorine group, respectively ($P<0.05$). The activity of CuZn-SOD in control group and fluorine plus zinc group significantly increased compared to fluorine group ($P<0.005$). Compared to control group, the activity of LDH in testis in fluorine group decreased ($P<0.05$). Fas expression in fluorine group was significantly increased compared to control group and fluorine plus group ($P<0.001$). Fas expression in fluorine plus zinc group was increased ($P<0.05$) compared to control group. **Conclusion.** Appropriate zinc can antagonize male reproductive toxicity of fluorine on molecular level by antagonizing lipid peroxidation, influencing reproduction endocrine, activity of enzyme, and Fas expression. [Life Science Journal. 2006;3(2):32-34] (ISSN: 1097-8135).

Keywords: fluorine; zinc; male reproductive toxicity; antagonistic mechanism; rat

Abbreviations: CuZn-SOD: superoxide dismutase; T: testosterone; LDH: lactate dehydrogenase

1 Introduction

It was indicated that zinc could antagonize male reproductive toxicity of excessive fluorine effectively[1-3]. Zinc could reduce absorption of fluorine, with the result of decreased fluorine in the body. Thus it antagonizes fluorotic toxity by improving the activity of CuZn-SOD and antagonizing lipid peroxidation[4]. There was few report about the antagonistic mechanism of zinc on male reproductive toxicity of excessive fluorine. The purpose of this study is to explore above question on molecular level and to provide reference data and scientific basis accordingly for prevention and treatment of fluorosis.

2 Materials and Methods

2.1 Animals

After one-week quarantine, thirty Wistar male rats weighted 55 to 65 grams were randomly allocated into three groups with ten rats each group: control group, fluorine group, and fluorine plus zinc group. All rats were provided by Henan Experimental Animals Center (Zhengzhou, Henan, China). The animals in control group, fluorine group, and fluorine plus zinc group were fed deionized water, solution of NaF (25 mg/kg), and solution of NaF (25 mg/kg) supplemented with $ZnSO_4$ (20 mg/kg), respectively. Each group was treated with solution of NaF or/and $ZnSO_4$ in the way of intragastric administration for six weeks.

2.2 Indexes of test

2.2.1 The level of testosterone in serum: Rats of the three groups were killed and blood serum was separated. The levels of serum testosterone were detected radioimmunochemically with ^{125}I-T kit. And this kit was provided by Beijing Beimiandongya Biological Technology Ltd.

2.2.2 Activity of CuZn-SOD in serum: Rats of the three groups were killed and blood serum was separated. The activity of CuZn-SOD was detected with SOD kit and colorimetric analysis method, and the kit was provided by Nanjing Jiancheng Biological Technology Ltd.

2.2.3 Activities of LDH in testis: Plasm of the testicle tissue was prepared, and the activities of LDH were examined by colorimetric analysis. The kit was provided by Nanjing Jiancheng Biological Technology Ltd.

2.2.4 Expression of Fas protein in testis: The levels of Fas protein expression in testis were detected immunohistochemically. Fas immunohistochemical kit was provided by Wuhan Boster Biologi-cal Technology Ltd, and the experiment was manipulated according to the direction. The process was operated as follows: One side of testis of rats was put into formaldehyde liquor and fixed for 48 h in normal temperature. Volume concentration of formaldehyde is ten percent. After deparaffinization, tissue sections were treated with 3% H_2O_2 for 10 min to eliminate endogenous peroxidase. These sections were then heated in 10 mM citrate buter (pH 6.0) for 5 min in a microwave oven three times to facilitate antigen retrieval and were incubated with BSA for 20 min at room temperature. Subsequently, the sections were incubated with rabbit anti-mouse Fas monoclonal antibody at 4°C overnight. Thereafter, the sections were incubated with biotinylated goat anti-rabbit IgG for 20 min and were incubated with SABC for 20 min at room temperature. The immunoreaction was visualized with di-aminobenzidine tetrahydrochloride (DAB). The sections were lightly counterstained with hematoxylin.

2.3 Statistical analysis

One-way analysis of variance (ANOVA) and non-parametric tests were used in the statistical test (SPSS13.0). There was significant difference when α was 0.05. Differences were considered significant when $P<0.05$.

Table 1. T level and CuZn-SOD activity of each group

Types of group	*n*	T (ng/dl)	CuZn-SOD (U/ml)
Control	10	22.40	37.50
Fluorine	10	16.00$^{\triangle}$	20.10$^{*\triangle}$
Fluorine plus zinc	10	38.20*	36.30
H		13.05	21.30
P		0.011	0.000

Note: * Compared with control group, $P<0.05$; $^{\triangle}$Compared with fluorine plus zinc group, $P<0.05$

3 Results

Table 1 showed the levels of T and activity of CuZn-SOD in each group. The levels of T in fluorine plus zinc group significantly increased compared to control group, and fluorine group, respectively ($P<0.05$). Activity of CuZn-SOD in control group and fluorine plus zinc group significantly increased compared to fluorine group ($P<0.005$). Table 2 showed the activity of LDH and the level of Fas expression in every group. Compared to control group, the activity of LDH in testis in fluorine group decreased ($P<0.05$). Fas expression in fluorine group significantly increased compared to control group and fluorine plus zinc group ($P<0.001$). Meanwhile, Fas expression in fluorine plus zinc group increased ($P<0.05$) compared to control group.

Table 2. LDH activity and Fas expression of each group

Types of group	*n*	LDH (U/g Prot)	Fas(%)
Control	10	1329.75±814.97	7.65
Fluorine	10	07.34±885.66*	36.65$^{*\triangle}$
Fluorine plus zinc	10	1220.46±812.24	15.35*
H		2.841	42.41
P		0.035	0.000

Note: * Compared with control group, $P<0.05$; $^{\triangle}$Compared with fluorine plus zinc group, $P<0.05$

4 Discussion

Six weeks later, experiment, the level of T, the activity of CuZn-SOD, and LDH decreased. Fas protein expression in germ cells increased. Thus all above indicated that model of fluorosis rats was successfully established.

Zinc plays an important role in synthesis, secretion, and metabolism of hormone. Yu[5] reported that the serum T in zinc deficiency group significantly decreased compared to that in control group. But it recovered to normal after supplementing zinc. Takihara[6] reported that it was better to cure male sterility and improve activity of sperm mobility using zinc and male hormone together. These results indicated that the level of testosterone in fluorine plus zinc group was higher than those of control group and fluorine group, and it was lower in fluorine group than that in control group. This explained that appropriate zinc make T increase by antagonizing reproductive endocrine disruptance of fluorine. Zinc is the critical component of CuZn-SOD. It could antagonize fluorosis by improving activity of CuZn-SOD and antagonizing lipid peroxidation, lessening damage induced by free radicals. This results showed that the activity of CuZn-SOD in fluorine group was lower than control group and fluorine plus zinc group. There was no significant difference of the activity between control group and fluorine plus zinc group. This indicated that zinc could antagonize fluorosis in the way of improving activity of CuZn-SOD and antagonizing lipid peroxidation. Zinc is important in maintaining functions of many enzymes because it is the component of them in the body and it has something with structure of enzymes. It was reported that appropriate zinc could improve the activity of LDH[7], which

was proved in this experiment. This experimental results showed that the activity of LDH in fluorine group significantly decreased compared to control group and fluorine plus zinc group. That's why the excessive fluorine could decrease the activity of LDH, while increased it after supplementing zinc. Therefore, zinc could antagonize reproductive toxicity of fluorine by influencing the activity of LDH.

Fas is a type I membrane protein that belongs to the tumor necrosis factor (TNF)/ nerve growth factor receptor family and mediates apoptosis upon binding to Fas ligand, a type II membrane protein which is a member of the TNF family[8-10]. Fas is the apoptosis-inducing gene[11]. When cell apoptosis improves, fas expression improves too. Zinc is apoptosis-restraining gene which can prevent many factors from apoptosis. Nodera M[12] reported that zinc deficiency induced apoptosis in germ cells. Li[13] reported that appropriate zinc made apoptosis in germ cells siganificantly. In this experiment, Fas protein expression in fluorine group was significantly higher than those in control group and fluorine plus zinc group, and it was significantly higher than that in control group. It was obvious that fluorine could improve Fas protein expression in germ cells, which indicated that fluorine could induce apoptosis in germ cells. Zinc could reduce Fas protein expression. It may antagonize reproductive damage of fluorine probably by restraining apoptosis which induced by Fas gene in germ cells. Yet, after supplementing zinc, Fas protein expression was still higher than that of control group. The reasons were probably that antagonism of zinc on Fas protein expression and apoptosis in germ cells was infinite, or the dosage of zinc was not enough which could not obviously antagonize apoptosis induced by Fas protein expression. It is necessary to make more research to find the mechanism.

In a word, appropriate zinc could antagonize the reproductive toxicity induced by fluorine in male rats on molecular level by antagonizing lipid peroxidation, influencing reproduction endocrine, activity of enzyme and Fas expression.

Correspondence to:

Liuxin Cui
Department of Environmental Health
College of Public Health
Zhengzhou University
Zhengzhou, Henan 450052, China
Telephone: 86-371-6665-8100
Email: clx@zzu.edu.cn

References

1. Zhang YZ, Liu KJ, Lu CR, et al. Study on distribution of zinc of preventive anti-fluorine agent in animal body. Ind Hlth& Occup Dis 2001; 127 (5):279-82.
2. Mazurek-Mochol M. Interaction between fluorine and zinc after long-term oral administration into the digestive system of rats. Ann Acad Med Stetin 2002;48: 75-83.
3. Krasowska A, Wlostowski T, Bonda E. Zinc protection from fluoride-induced testicular injury in the bank vole (Clethrionomys glareolus). Toxicol Lett 2004; 147 (3): 229-35.
4. Xia T, Wang AG, Yu RA, et al. Experimental study on antagonistic effects of Se-Zn agent on chronic fluorosis. Chinese Journal of Endemiology 2001;20(3):180-2.
5. Yu XQ, Chen SL, Xie HW, et al. The effect of zinc deficiency and zinc replenishment on testicle and testosterone in rats. Practical Preventive Medicine 2004; 11 (2):219-20.
6. Takihara H, Cosentino MJ, Cockett AT. Effects of low-dose androgen and zinc sulfate on sperm motility and seminal zinc levels in infertile men. Toxicol Lett 2004; 147(3):229-35.
7. Leng J, Zhu RJ, Ma L. Influence of zinc in forage on serum enzyme activities. Chinese Forage 2004;(9):15-6.
8. Eguchi J, Koji T, Nomata K, et al. Fas-Fas ligand system as a possible mediator of spermatogenic cell apoptosis in human maturation-arrested testes. Hum Cell 2002, 15 (1): 61-8.
9. Li HJ, Guo YL. Fas/FasL and testis. Reproduction and contraception(in Chinese) 2000;20(1): 7-11.
10. Ogis, Tianji N, Yokoyama M, et al. Involvement of Fas in the apoptosis of mouse germ cells induced by experimental cryptochidism. Urol Res 1998; 26(1): 17-21.
11. Tang WF, Jiang JM. Fas system, apoptosis and acute pancreatitis. HuaXi medicine 2005;20(1):179-80.
12. Nodera M, Yanagisawa H, Wada O. Increased apoptosis in a variety of tissues of zinc-deficient rats. Life Sci 2001; 69(14):1639-49.
13. Li JS, Wang C, Wang J, et al. The influence of zinc supplementation on apoptosis of old rats' testicular cells its nitric oxide synth activity. Chinese Journal of Agedness 2000; 20(2):93-4.

Received March 9, 2006