THE INFLUENCE OF FLUORIDE ON THE CONTENT OF TESTOSTERONE AND CHOLESTEROL IN RAT

Z L Zhao, N P Wu and W H Gao
Ningxia, China

SUMMARY: Fifty four Wistar male rats were randomly divided into three groups, drinking water containing 0.6 mg/L (control group), 100 mg/L, and 200 mg/L sodium fluoride, respectively. Rats were killed at the second, fourth and sixth weeks after experiment initiation, respectively. The levels of serum testosterone, testis cholesterol, and hepatic tissue cholesterol were determined. Results showed that the serum testosterone level had decreased with time in rats drinking water containing 100 and 200 mg/L fluoride. While testis cholesterol level did not change, it was significantly decreased in the liver at the fourth and sixth week when compared with the control group. Results suggest that fluoride may have some harmful effects on the reproductive system in male rats.

Key words: Cholesterol; Fluoride; Rat; Testosterone.

Introduction

Fluoride intoxication of non-skeletal tissue has recently gained increasing attention. Fluoride is a toxin that can damage internal organs. The present study was undertaken to investigate the influence of fluorine on the reproductive system by determining the levels of serum testosterone, testis cholesterol and hepatic tissue cholesterol in male rats.

Materials and Methods

Fifty four healthy male Wistar rats weighing 165±25 g at the beginning of the experiment were obtained from the Laboratory Animal Center of Ningxia Medical College. The rats were randomly divided into three equal groups. All rats were housed in the same room with free access to food and water. The control group was given boiled tap water in which the fluoride concentration was 0.6 mg/L, the experimental groups were given drinking water containing 100 and 200 mg/L sodium fluoride (NaF), respectively. At the second, fourth and sixth weeks after initiation the rats were killed by decapitation between 900 h and 1000 h. Trunk blood was collected, and the serum was separated and frozen at -20°C for testosterone assay.

The testis and hepatic tissue (approximately 0.5 g) were quickly removed, weighed, and homogenized in 5 ml of 1.15% KCl - 1 mMol/L Tris-HCl (pH 7.0) buffer, centrifuged (1500 rpm, 20 min), and then assayed for cholesterol.

Serum samples were assayed for testosterone by radioimmunoassay (RIA). All samples were determined during one assay. Testis and hepatic tissue samples were assayed for cholesterol by the o-phthalaldehyde coloration method. Tissue protein content was determined by the Coomassie brilliant blue dye combining method. Student's T test was used to determine significance of differences between groups at the same period.

Department of Preventive Medicine, Ningxia Medical College, 750004 China.
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Results

Serum testosterone levels increased significantly at the 2nd week in the groups of rats given water containing 100 and 200 mg/L, respectively. They then decreased between the fourth and sixth weeks, and were not significantly different from the controls (Table 1).

Table 1. Serum testosterone concentration (ng/ml)

Group	2 weeks (n=6)	4 weeks (n=6)	6 weeks (n=6)
0.6 mg/L	1.23 ± 0.66	2.05 ± 0.96	2.16 ± 2.00
100 mg/L	3.03 ± 1.21*	1.94 ± 1.06	1.73 ± 1.20
200 mg/L	2.40 ± 0.91*	2.01 ± 1.71	1.77 ± 0.91

Table 2. Testis cholesterol concentration (μg/mg protein)

Group	2 weeks (n=6)	4 weeks (n=6)	6 weeks (n=6)
0.6 mg/L	7.33 ± 1.22	8.61 ± 1.38	7.72 ± 1.12
100 mg/L	8.26 ± 1.00	7.65 ± 1.58	7.69 ± 1.22
200 mg/L	8.04 ± 0.81	7.31 ± 0.83	7.60 ± 1.10

Table 3. Hepatic tissue cholesterol concentration (μg/mg protein)

Group	2 weeks (n=6)	4 weeks (n=6)	6 weeks (n=6)
0.6 mg/L	10.41 ± 2.59	7.65 ± 1.79	6.59 ± 0.59
100 mg/L	10.27 ± 0.96	5.76 ± 0.91*	5.76 ± 0.39*
200 mg/L	9.04 ± 1.04	6.01 ± 0.81*	5.74 ± 0.67*

Values are the mean ± SD
* $p < 0.05$

Discussion

The data presented in this study show that in experimental rats, serum testosterone levels increased at the second week, but declined at the fourth and sixth week. One most important physiological function of testosterone is to promote cell protein synthesis. Fluoride can inhibit cell protein synthesis.[1-3] A possible explanation of the results of the present study is that fluoride initially inhibits the cell protein synthesis and consequently testosterone secretion increases. Later, fluoride inhibits the testosterone synthesizing mechanism, thus decreasing levels of testosterone. The phenomenon may be caused by body stress reaction, but this has yet to be investigated. As the period of drinking fluoridated water is prolonged, the content of fluoride in experimental rats' bodies increased, and serum testosterone levels

decreased. Although during the period of the present study serum testosterone level decreases did not show significance as compared with the control group, the correlation coefficient between the time of drinking fluoridated water and serum testosterone levels was -0.9307 (100 mg/L group) and -0.9917 (200 mg/L group), which suggests that fluoride can damage the reproductive system and decrease the secretion of testosterone.

Cholesterol is a material used for testosterone synthesis. The liver is the most efficient organ of the body in synthesizing cholesterol. It has been reported that fluoride causes injury to hepatic tissue.[4-6] Thus the liver's ability to synthesize cholesterol would be decreased, and the cholesterol level would decrease. This might induce a decrease in testosterone synthesis in the testis. Therefore, we determined the cholesterol levels of testis and hepatic tissue at the same time. Results showed that the cholesterol levels of hepatic tissue decreased significantly at the fourth and sixth week. But the testis cholesterol levels remained constant through the experimental period. These results support those reported previously concerning the fluoride-induced changes to liver.[7] Since in the present study the decrease of liver cholesterol did not induce changes in testis cholesterol, fluoride may injure testis Leydig cells directly.

It has been reported that fluoride has an inhibiting effect on protein synthesis and activity of many enzymes, and also damages cell membranes directly.[1] It is tempting to speculate that fluoride first inhibits protein synthesis and enzyme activity as well as damages testis cells directly, and only then influences the synthesis and secretion of testosterone.

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