EFFECTS OF SELENIUM ON SERUM LIPIDS AND ENZYME ACTIVITIES IN FLUORIDE-INTOXICATED RATS

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SUMMARY: Male Wistar rats were exposed to sodium fluoride (20 mg F⁻/kg bw/24 hr) orally during 3 months. Sodium selenite (5 or 10 µg elemental Se/kg bw/24 hr) was simultaneously administered to some of the animals. Changes in serum lipids and enzyme activities caused by fluoride intake were less pronounced in rats receiving selenium.

Keywords: Food-borne fluoride, Rat intoxication, Selenium, Serum enzymes, Serum lipids.

INTRODUCTION

Selenium is generally recognized as an important antioxidant with numerous biological functions. In spite of intense research during recent years, the role of this microelement needs further elucidation. We therefore decided to use the sodium fluoride (NaF) model of chronic intoxication in rats to determine whether selenium is able to prevent some of the accompanying biochemical changes. Our previous report on the activity of some oxidative and lysosomal enzymes in NaF intoxication pointed to the protective action of selenium.¹

MATERIALS AND METHODS

Male Wistar rats (6-7 weeks old) with an initial body weight of approximately 200 g were divided into 6 groups of 10 animals each and treated as follows:

- Group I – Control (no F, no Se);
- Group II (Se-5) – 5 µg elemental Se/kg body weight/24 hr;
- Group III (Se-10) – 10 µg elemental Se/kg body weight/24 hr;
- Group IV (NaF) – 20 mg NaF/kg body weight/24 hr;
- Group V (NaF+Se-5) – 20 mg NaF + 5 µg elemental Se/kg body weight/24 hr;
- Group VI (NaF+Se-10) – 20 mg NaF + 10 elemental µg Se/kg body weight/24 hr.

Sodium selenite (Na₂SeO₃) and sodium fluoride (NaF) were added to standard rat chow as pellets. Chow and water were given ad libitum. The

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animals were weighed every 14 days. After 3 months the animals were anesthetized with ether and blood was drawn by cardiac puncture.

The following biochemical tests in serum were performed:

- Enzyme activities: alanine (AIAT) and aspartate (AspAT) aminotransferases, alkaline phosphatase (AP), γ-glutamyl transferase (γ-GTP);
- Bilirubin;
- Lipids: total lipids, triglycerides and total cholesterol, cholesterol in LDL, and HDL fractions.

Statistical analyses were done using Student’s t-test for unpaired results and taking p<0.05 as the level of significance.

RESULTS

No statistically significant differences in weight gain between the control and experimental groups of rats were detected.

Serum enzyme activities (Figures 1a and 1b):

Alanine transaminase (AIAT): Intoxication increased the activity of AIAT by 27%, whereas supplementation with 5 µg Se/kg bw/24 hr reduced the activity by 21% in comparison with the NaF-intoxicated group. The higher dose (10 µg elemental Se) was without statistically significant effect.

Aspartate transaminase (AspAT): The activity of AspAT was 32% higher in the NaF-intoxicated group. Compared with this group, the activity was 24% lower in rats given 5 µg Se/kg bw/24 hr. No statistically significant difference was observed when the dose of Se was doubled.

γ-Glutamyl transferase (γ-GTP): A rise of 80% in the γ-GTP activity was observed in rats exposed to NaF. This increase was 37% less with the higher dose of Se, but with the lower dose the activity was actually 13% less than the control.

Alkaline phosphatase (AP): The activity of this enzyme in rats exposed to NaF was 72% higher than in control animals. With Se the increase in activity was significantly less with the higher dose but not with the lower dose.

Bilirubin in serum (Figure 1b):

No statistically significant differences in bilirubin levels between the control and remaining groups were observed.

Serum lipids (Figures 2a and 2b):

Total lipids: The concentration of total lipids in rats exposed to NaF rose by 32% as compared with controls. Administration of 5 µg Se/kg bw/24 hr resulted in a 17% reduction compared to the exposed group. No statistically significant difference was found with the higher dose of Se.
Figure 1. Influence of selenium intake on some serum enzyme activities and the bilirubin level in rats chronically exposed to sodium fluoride (control = 100 %).

*Statistically significant in comparison with the control group (p < 0.05).
**Statistically significant in comparison with the exposed group (p < 0.05).

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Figure 2. Influence of selenium intake on serum lipid levels in rats chronically exposed to sodium fluoride (control = 100%).

*Statistically significant in comparison with the control group (p < 0.05).
**Statistically significant in comparison with the exposed group (p < 0.05).
Triglycerides: Triglyceride levels rose by 45% in the NaF-intoxicated group. In comparison with this group, 5 µg Se/kg bw/24 hr reduced triglyceride levels by 36%. When the higher dose of Se was administered the levels were slightly higher than in NaF-intoxicated animals.

Cholesterol: Exposure to NaF increased total and LDL cholesterol by 69% and 102%, respectively, and reduced HDL cholesterol by 19%. Administration of 5 µg Se/kg bw/24 hr reduced total LDL and cholesterol by 38% and 39%, respectively, and increased HDL cholesterol by 27%, as compared with the NaF group. The reduction in total and LDL cholesterol with the higher dose of Se was 44% and 17%, respectively, without any significant change in HDL cholesterol.

DISCUSSION

The extent of toxic damage to hepatocytes can be revealed by measuring activities of cytoplasmic and mitochondrial enzymes, the former being released before the latter as damage progresses. Elevated enzyme activities in our exposed rats unequivocally reflect hepatic injury by NaF. Higher activities of γ-GTP are indicative of cholestasis caused by liver damage. Although bilirubin levels in exposed rats were elevated, the difference was not statistically significant as compared with the control group. However, Lee observed mild hyperbilirubinemia in patients with Gibert’s disease that was dependent on fluoride intake from drinking water.

One of the chief mechanisms responsible for fluoride toxicity involves the binding of magnesium ions, leading to inhibition of Mg-dependent enzymes, such as alkaline phosphatase. In the present study, the activity of this enzyme increased. A similar observation was made previously. More relevant conclusions concerning this enzyme could be drawn from levels of the bone isoenzyme.

Experimental work on lipid metabolism during exposure to fluoride is limited and the results are conflicting. Vatassery et al and Dousset et al have observed reduced levels of cholesterol and total lipids in serum and liver in guinea pigs during the first phase of intoxication. Mąkowska et al observed elevated levels of cholesterol and reduced levels of β-lipoproteins in subjects chronically exposed to high fluoride concentrations. Finally, Chinoy et al did not detect any significant change in lipid levels during NaF exposure. Nevertheless, changes in serum triglyceride and cholesterol levels during exposure to fluorine compounds appear to result from alterations in enzyme activities, particularly triglyceride lipase, some unspecific esterases, and pyrophosphatase, thereby causing inhibition of fatty acid oxidation.

Selenium and its role in metabolism have recently become the focus of numerous studies. This microelement is indispensable for normal function of...
the organism. Se is found in enzymes protecting the cell against free radicals and exhibits immunomodulatory, anti-inflammatory, anti-viral, and anti-tumor properties. It takes part in cell growth,\textsuperscript{17} stabilizes cellular membranes,\textsuperscript{18} affects DNA replication,\textsuperscript{19} and alters cellular immunity.\textsuperscript{20} Thus, both deficiency and excess of Se are harmful, and the span between the therapeutic and toxic dose is narrow.

Various biological interactions between selenium and fluorine are currently under investigation. Increased intake of F is accompanied by greater excretion of Se,\textsuperscript{21,22} and higher dietary levels of fluorine in residents of selenium-rich areas are associated with smaller reduction in caries incidence.\textsuperscript{23,24} Some reports on the interactions between selenium and fluorine have led to equivocal and sometimes conflicting conclusions. Several authors have failed to observe any interaction, while others advocate a beneficial effect of Se on the energy metabolism of skeletal muscles and histological lesions in fluorosis.\textsuperscript{1,25,26} Yu et al\textsuperscript{27} reported that increased dietary intake of F\textsuperscript{-} reduced the content of Se in the liver and prevented necrosis of hepatocytes caused by selenium compounds. Therapeutic doses of Se lead to normalization of intracellular enzyme activities,\textsuperscript{28} and Se is indispensable for normal pancreatic function.\textsuperscript{29} The present results confirm the beneficial effect of selenium in rats chronically exposed to sodium fluoride.

In our study, the activity of aminotransferases and γ-GTP and levels of triglycerides, total lipids, and LDL cholesterol in rats given 5 µg Se/kg bw/24 hr were reduced, while HDL cholesterol was increased. When the dose of Se was doubled, protection against NaF was less evident. It follows that in order to avoid side effects, the dosage of Se should be carefully controlled, particularly during long-term therapy.

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