

LIVER MORPHOLOGY AND HISTOCHEMISTRY IN RATS RESULTING FROM INGESTION OF SODIUM SELENITE AND SODIUM FLUORIDE

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SUMMARY: A morphological and histochemical assessment was made of the effect of sodium selenite and sodium fluoride on oxidative and lysosomal enzyme activity in the liver of male rats. The compounds were administered separately and together for a period of three months. Oxidative enzyme activity of α -glycerophosphate dehydrogenase (α -GIDH) and succinate dehydrogenase (SDH) increased with sodium selenite and decreased with sodium fluoride and with both compounds together. Sodium selenite in a dose of 10 μ g/kg body weight and sodium fluoride in a dose of 20 mg/kg body weight caused an increase in the activity of acid phosphatase (AcP), while the combined dose caused a decrease in the activity of this enzyme.

Keywords: Acid phosphatase, α -Glycerophosphate dehydrogenase, Histochemistry, Lysosomal enzymes, Male rats, Oxidative enzymes, Rat liver, Sodium fluoride, Sodium selenite, Succinate dehydrogenase.

INTRODUCTION

The liver is one of the major animal organs where selenium is deposited.¹ The principal role of selenium is associated with the control of lipid peroxidation because this trace element is a component of selenoenzymes contributing to the antioxidant system.²⁻⁵ Under certain conditions selenium is an immunostimulative factor.⁶ Both deficiency and oversupply of selenium in a diet are associated with the occurrence of clinical symptoms including Keshan disease and Kashin Beck disease.⁷ On the other hand, fluorine is a well known inhibitor of numerous enzymes involving carbohydrate and cell-energy metabolism.^{8,9} Increasingly, its role as an activator of numerous enzymes is being reported.¹⁰

Previous studies on interactions between selenium and fluorine have not yielded conclusive results.⁷ Some researchers have not found any interactions between these microelements.^{11,12} Others have observed a protective effect of selenium on energy metabolism of skeletal muscles in the course of fluorosis.¹³

The aim of the present study was to determine morphological changes and the activity of selected oxidative and lysosomal enzymes in the liver of rats fed a diet containing selenium and fluorine compounds.

MATERIALS AND METHODS

Our study was conducted on 66 eight-week-old Wistar male rats divided into six groups of eleven each. Group I (control) was fed standard feed and water *ad libitum*; group II was fed sodium selenite 5 μ g/kg body weight/day; group III was fed sodium selenite 10 μ g/kg body weight/day; group IV was fed 20 mg/kg of sodium fluoride (NaF); group V was fed with a combined dose of 5 μ g/kg of sodium selenite and 20 mg/kg of NaF; and group VI was fed 10 μ g/kg of so-

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dium selenite and 20 mg/kg of NaF. Animals of the exposure groups (II to VI) were fed daily on an empty stomach a ration of feed containing doses of NaF and/or sodium selenite. Each rat was fed individually. Following the above procedure the rats were given both water and feed *ad libitum*. After 3 months the animals, weighing 500 g on average, were decapitated under ether anesthesia.

Liver samples intended for histological examination were fixed in Bouin's solution, embedded in paraffin, sectioned, and stained with Mayer's hematoxylin and aqueous eosin. For histochemical analysis liver samples were promptly frozen and subsequently cut on a cryostat into 10- μ m sections. Frozen (unfixed) liver sections were studied for the following enzymes: α -glycerophosphate dehydrogenase (α -G1DH) according to Hess *et al.* (EC 1.1.1.95) using sodium α -glycerophosphate, succinate dehydrogenase (SDH) according to Nachlas, using sodium succinate as the substrate (EC 1.3.99.1), and acid phosphatase (AcP) (EC 3.1.3.2) using azo-dye coupling method with α -naphthol phosphate.¹⁴

RESULTS

Pathomorphological changes: Livers of control rats (group I) had a regular histological structure (Figure 1) with a characteristic pattern of hexagonal lobules. In the livers of group II no major morphological changes were observed compared to the control group, except for small sporadic infiltrations of mononuclear cells in portal canals and weak activation of Kupffer cells. In the livers of group III a distinct swelling of Kupffer cells was observed in dilated sinusoidal vessels, mainly in the proximity of portal fields. Necrotic areas comprising single groups of hepatocytes were occasionally observed. In all experimental animals of group IV relatively abundant infiltrations of mononuclear cells occurred in portal canals (Figure 2). Sporadically, there were areas of necrosis within individual lobules. The individual necrotic hepatocytes were surrounded by lymphocytes. The changes were accompanied by activation and swelling of Kupffer cells in widened sinusoidal vessels.

In the livers of group V rats there were foci of necrosis affecting groups of hepatocytes, most often in the intermediate zone of the lobules. Occasionally there were small infiltrations of mononuclear cells in portal canals and limited infiltrations of lymphocytes around the single necrotic hepatocytes. In the livers of group VI rats an obliteration of the laminar structure was observed. The parenchyma showed extensive areas of necrosis covering individual lobules. Sometimes infiltrations of mononuclear cells of variable intensity were observed in portal canals and around central veins. Necrotic hepatocytes were replaced by an infiltration of lymphocytes (Figure 3).

Histochemical study

α -Glycerophosphate dehydrogenase (α -G1DH): In the liver of the control group a moderate or rarely strong reaction to α -G1DH was seen in the form of small granules of formazan (Figure 4). Slightly weaker activity of this enzyme was observed in the central zone compared to the intermediate and peripheral

zones of the lobules. In group II a strong activity of the enzyme was observed, mainly in the peripheral and intermediate zones (Figure 5). Increased activity of α -GIDH occurred in the liver of rats of group III in the form of a strong or very strong reaction in all zones of the lobules. In groups IV, V, and VI a distinct decrease in the activity of this enzyme was noted compared to the control group. A weak activity of this enzyme was observed in groups IV and VI. The granules of the reaction product were localized mainly in the intermediate and peripheral zones of the lobules. Rat livers of group V exhibited moderate or weak reaction to α -GIDH (Figure 6). The intensity of the reaction gradually grew from the central vein toward the peripheral zone of the lobules.

Succinate dehydrogenase (SDH): The liver of control rats showed moderate activity of SDH (Figure 7). Formazan granules were almost evenly distributed within the lobules, with the exception of the peripheral zone which exhibited slightly stronger reaction. Increase in SDH activity was observed in the livers of animals fed selenium (groups II and III). A strong reaction to SDH was observed in the livers of group II rats, whereas the activity of SDH was weaker in the area of the central vein. In group III, a strong or very strong activity of SDH was observed, mainly in the periphery of the liver lobules, and it gradually weakened toward the central zone (Figure 8). A drastic decrease in this enzyme activity occurred in the rats fed NaF. The liver of rats of group IV demonstrated a weak activity of SDH (Figure 9). In the area of the central vein and in the portal triads, there were often negative reactions to this enzyme. The product of the reaction was located mainly in the intermediate zone of the lobules. Slightly stronger reaction to SDH was observed in group V, in which the activity of this enzyme was weak or moderate. The granules of the reaction product were localized predominately in the intermediate zone, whereas the peripheral zone showed the weakest reaction. A weak reaction to SDH occurred in the liver of group VI rats. The presence of active enzyme was observed mainly in the central and intermediate zones of the lobules.

Acid Phosphatase: In control group I as well as in experimental group II, a moderate activity of AcP was observed (Figure 10). In the control group a slightly weaker activity of the enzyme was visible in the intermediate zone of the lobules, whereas in group II the reaction product was evenly distributed in the liver lobules. A strong reaction to AcP in a form of larger granules of the reaction product was observed in groups III and IV (Figure 11). In both these groups the central zone of the lobules exhibited a slightly weaker reaction to this enzyme compared to the intermediate and peripheral zones. A diminished reaction to AcP was observed in group V, wherein a weak or moderate reaction to AcP occurred. Only the intermediate zone of the lobules demonstrated a slightly stronger reaction to this enzyme. A similarly weak reaction was visible in the animals of group VI (Figure 12) in which the granules of reaction product were localized evenly within the lobules.

α -GIDH, SDH, and AcP activities in the control and experimental rat livers after 3 months are shown in Figure 13.

Figure 1

Liver of a control rat
Group I (x 140, HE)

Figure 2

Liver of a rat
treated with NaF
Group IV (x 140, HE)

Figure 3

Liver of a rat treated with
NaF and sodium selenite
Group VI (x 140, HE)

Figure 4

A moderate α -GIDH reaction
in the liver of a control rat
Group I (x 140)

Figure 5

A strong α -GIDH reaction
in the liver of a rat treated
with sodium selenite
Group II (x 140)

Figure 6

A weak or moderate
 α -GIDH reaction in the
liver of a rat treated with
NaF and sodium selenite
Group V (x 140)

Figure 7

A moderate SDH reaction
in the liver of a control rat
Group I (x 140)

Figure 8

A strong or very strong SDH
reaction in the liver of a rat
treated with sodium selenite
Group III (x 140)

Figure 9

A weak or moderate
SDH reaction in the
liver of a rat treated with
NaF and sodium selenite
Group V (x 140)

Figure 10

A moderate AcP reaction
in the liver of a control rat
Group I (x 140)

Figure 11

A strong AcP reaction
in the liver of a rat
treated with NaF
Group IV (x 140)

Figure 12

A weak AcP reaction in
the liver of a rat treated with
NaF and sodium selenite
Group VI (x 140)

Figure13. α -GIDH, SDH and AcP activities in the control and experimental rat liver
I – control group, II – VI – experimental groups
1- weak reaction, 2- moderate reaction, 3- strong reaction, 4- very strong reaction

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DISCUSSION

The present study revealed that significant changes occurred in the intensity of reactions to certain enzymes in the liver of rats fed diets containing compounds of selenium and fluorine. Fluorine administered as NaF in a dose of 20 mg/kg body weight (group IV) caused pathomorphological changes of variable intensity. The changes ranged from degeneration to necrosis of hepatocytes as also described by the other authors.^{15,16} This picture of pathomorphological changes is undoubtedly connected with a substantial activity drop of the oxidoreductases studied (α -GIDH, SDH). Activity decrease of SDH and other enzymes of the Krebs cycle by fluoride ions in the liver of rats and hamsters has been demonstrated in histochemical and biochemical studies.¹⁷⁻²⁰ Histochemical studies on the effect of fluoride in the skeletal muscles of rats also show, besides a drastic decline of SDH activity, decrease in the activity of cytochrome c oxidase and Mg^{+2} -ATPase.¹³ The ultrastructural study showed that fluoride ions caused disintegration and swelling of mitochondrial cristae, which undoubtedly leads to anomalies in the respiratory metabolism.^{13,21,22}

Results of the present study demonstrated that sodium fluoride and sodium selenite administered jointly (group V) caused a smaller decrease in the activity of the enzymes studied than in the case of fluoride alone. On the other hand, a two-fold increase of selenium administered jointly with fluorine resulted in substantially less protective effect (group VI). Selenium alone caused a distinct increase of activity in the enzymes of aerobic and anaerobic metabolic pathways. The increase was in direct proportion to the size of the dose (groups II and III). The morphological study of the rat livers of those experimental groups revealed relatively minor regressive changes. A similar picture of pathomorphological changes in the liver under influence of selenium has been described by the other authors.^{4,23}

Our results indicate that selenium, in sufficient doses, may exert a certain protective impact on the activity of oxidative enzymes. These observations are in accord with those of Pang *et al*¹³ on *in vivo* and *in vitro* studies of skeletal muscles of rats treated with fluoride and selenium. These authors associate the protective effect of selenium with improved stability of mitochondrial membranes as a result of decreased lipid peroxidation. Histochemical analysis in our study revealed that this activity increase of the oxidative enzymes under influence of selenium was connected with intensification of the reactions, mainly in the peripheral and intermediate zones of the lobules. The zone adjacent to the portal triads is associated with metabolic activity of the hepatocytes in the processes of gluconeogenesis and cellular respiration. As is known, individual zones of liver lobules exhibit structural and metabolic heterogeneity.²⁴ The heterogeneity of liver lobules is a consequence of the direction of blood flow, which results in variable oxygen supply to hepatocytes in different zones. Decreased activity of oxidative enzymes under the influence of combined administration of fluorine and selenium was greatest in the zone of the central vein and in the peripheral area of the lobules.

The present study also revealed an increase of AcP activity in the livers of animals treated with either selenium and fluorine (groups III and IV). The highest increase was observed in the zone of hepatocytes adjacent to portal triads, which was probably associated with the presence of higher number of Kupffer cells in the first zone of hepatic acini. Probably the increase of AcP activity in both parenchymal and non-parenchymal cells is associated with intensification of intracellular catabolism under the influence of selenite and fluoride ions. Increased activity of AcP from fluoride ions has been demonstrated in the liver of squirrel monkeys²⁵ and in the intestines of mice,²⁶ whereas a decrease has been observed in the livers of mice and rats.^{17,27}

It can be concluded therefore, that discrepancies related to AcP activity levels under influence of fluorine may stem from different fluorine doses, compound used, organ studied, species of experimental animal, duration of the experiment, and other factors.² In our experimental groups where fluorine and selenium were administered together (groups V and VI), a decrease in AcP activity in the liver was observed, which is undoubtedly associated with the presence of regressive changes occurring more intensively in those experimental groups.

REFERENCES

- 1 Park YC, Whanger PD. Toxicity, metabolism and absorption of selenite by isolated rat hepatocytes. *Toxicology* 1995;100:151-62.
- 2 Jendryczko A, Drózd M. Selenoenzymes as factors protecting against oxygen stress. *Wiad Lek* 1993;46:62-5.
- 3 Danch A, Magner-Wróbel K, Drózd M, Taborek M. Changes of some metals content in the plasma and liver of rats treated with procainamide and selenium. *Bromat Chem Toksykol* 1994;27:67-71.
- 4 Turan B, Zaloglu N, Koc E, Saran Y, Akkas N. Dietary selenium- and vitamin E-induced alterations in some rabbit tissues. *Biol Trace Elem Res* 1997; 58:237-53.
- 5 Posiełężna B, Drózd M, Jendryczko A. Protective effect of sodium selenite on liver inflammatory state of rats intoxicated with nitroso-alpha-naphthol. Part I. Activities alterations of glutathione peroxidase, superoxide dismutase, and catalase. *Bromat Chem Toksykol* 1993;26:256-65.
- 6 Jendryczko A. Modulatory properties of selenium in immune processes. *Wiad Lek* 1994;47:198-202.
- 7 Whanger PD. China, a country with both selenium deficiency and toxicity: some thoughts and impression. *J Nutr* 1989;119:1236-39.
- 8 Gumińska M, Sterkowicz J. Effect of sodium fluoride on glycolysis in human erythrocytes and Ehrlich ascites tumour cells *in vitro*. *Acta Biochim Pol* 1976;23:285-91.
- 9 Zebrowski EJ, Suttie JW. Glucose oxidation and glycogen metabolism in fluoride-fed rats. *J Nutr* 1988;66:267-72.
- 10 Chlubek D, Machoy Z. Significance of the effect of fluorine dose on enzymes activity in *in vivo* and *in vitro* studies. *Bromat Chem Toksykol* 1989;22: 235-45.
- 11 Hadjimarkos DM. Selenium toxicity: effect of fluoride. *Experientia* 1969; 25:485-6.

- 16 Kołodziejczyk, Put, Grzela
- 12 Shearer TR, Ridlington JW. Fluoride-selenium interaction in the hard and soft tissues of the rat. *J Nutr* 1976;106:451-6.
- 13 Pang YX, Guo YQ, Zhu P, Fu KW, Sun YF, Tang RQ. The effects on fluoride, alone and in combination with selenium, on the morphology and histochemistry of skeletal muscle. *Fluoride* 1996;29:59-62.
- 14 Pearse AGE. *Histochemistry. Theoretical and applied.* Vol.2. Third edition. Edinburgh and London: Churchill Livingstone; 1972.
- 15 Kour K, Koul ML, Koul RL. Histological changes in liver following sodium fluoride ingestion. *Fluoride* 1981;14:119-23.
- 16 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 XXIIInd ISFR Conference Abstracts.
- 17 Singh M, Kanwar KS. Effect of fluoride on tissue enzyme activities in rat: biochemical and histochemical studies. *Fluoride* 1981;14:132-41.
- 18 Sullivan WD. The *in vitro* and *in vivo* effects of fluoride on succinic dehydrogenase activity. *Fluoride* 1969;2:168-75.
- 19 Grucka-Mamczar E, Machoy Z, Tarnawski R, Birkner E, Mamczar A. Influence of long-term fluoride administration on selected parameters of rat blood serum and liver function. *Fluoride* 1997;30:157-64.
- 20 Chinoy NJ, Mehta D. Beneficial effects of the amino acids glycine and glutamine on testis of mice treated with sodium fluoride. *Fluoride* 1999;32(3):62-70.
- 21 Malmqvist E, Löw H. Electron microscopic changes in mitochondria of the intestinal epithelium and the liver in mice and D-avitaminotic rats after peroral distribution of sodium fluoride. *J Ultr Res* 1969;29:276-80.
- 22 Chinoy NJ, Patel D. Ultrastructural and histopathological changes in ovary and uterus of fluorotic mice and reversal by some antidotes. *Fluoride* 1998;31:S27 XXIIInd ISFR Conference Abstracts.
- 23 Szarek J, Zasadowski A, Fabczak J, Spodniewska A. Effect of sodium selenite and fenitrothion on pathomorphological pattern of liver and kidneys in rats. *Arsen i selen w środowisku – problemy ekologiczne PAN* 1994;149-56.
- 24 Kamiński M, Starek A. Structural and metabolic heterogeneity of liver – toxicologic implications. *Acta Pol Toxicol* 1994;2 Suppl 1:55-62.
- 25 Manocha SL, Warner H, Olkowski ZL. Cytochemical response of kidney, liver and nervous system to fluoride ions in drinking water. *Histochem J* 1975;7:343-55.
- 26 Sondhi H, Gupta ML, Gupta GL. Intestinal effects of sodium fluoride in Swiss albino mice. *Fluoride* 1995;28:21-4.
- 27 Bogin E, Abrams M, Avidar Y, Israeli B. Effect of fluoride on enzymes from serum, liver, kidney, skeletal and heart muscles of mice. *Fluoride* 1976;9:42-6.

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