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The influence of vitamin E and methionine on the activity of enzymes and the morphological picture of liver of rats intoxicated with sodium fluoride

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ABSTRACT

The aim of this study was to investigate the effects of vitamin E and methionine on the activity of enzymes regulating carbohydrate metabolism and enzymes associated with glutathione as well as to examine the morphology of the liver in rats exposed to sodium fluoride.

The study was conducted in 18 male rats of Wistar strain. The rats were divided into three groups: a control group, which received distilled water and two experimental groups, which received sodium fluoride (10 mg/kg of body mass/24 h) in water solution. Animals in the second experimental group received 3 mg of vitamin E/rat/24 h and 2 mg methionine/rat/24 h. The experiment lasted 35 days. In supernatants obtained after homogenization of rat liver slices, the activity of the following enzymes was assayed: fructose-1,6-biphosphate aldolase (ALD) malate dehydrogenase (MDH), lactate dehydrogenase (LDH), sorbitol dehydrogenase (SDH) the activity of glutathione peroxidase (GPx), glutathione transferase (GST) and glutathione reductase (GR). Pathomorphological evaluation was conducted on preparations made by standard paraffin method, followed by staining with hematoxylin and eosin. The administration of antioxidants counteracted changes in the activity of the enzymes and the morphological abnormalities of the liver induced by NaF. Antioxidants may be important in preventing toxicity of fluoride compounds.

1. Introduction

Fluorine is a trace element necessary for the proper organism function. Owing to its high reactivity, fluorine is found in natural environment only in the bound form. It is the most electronegative element and one of the strongest oxidants (Hordyjewska and Pasternak, 2004). Among all halogens fluorine has the lowest molecular mass and the smallest ionic radius, thus it easily penetrates into the cells and changes their chemical properties by binding with other elements and compounds. Fluorine exhibits the strongest affinity for: calcium, magnesium, manganese, molybdenum, iron, aluminum, copper and zinc (Machoy, 1987). In the conditions exceeding the toxic dosage fluorine exerts the harmful effects on the organism. Fluoride ions can affect the enzyme system. They may directly or indirectly modulate the enzyme activity by forming complexes with metals incorporated into enzyme molecules (Pawłowska-Góral et al., 1998). In this way fluorides interfere with the course of metabolic processes involving carbohydrates, lipids and proteins. Fluorides inhibit or, less frequently, activate important enzymes involved in many metabolic pathways. These are primarily enzymes involved in glycolysis and the Krebs cycle. In addition, fluorides inhibit fatty acid oxidation and reduce the activity of pyruvate dehydrogenase, which reduces the amount of acetyl-CoA in the cells and decreases cholesterol synthesis. Fluorides can also inhibit the activation of amino acids during translation. Sodium fluoride (NaF) negatively regulates the concentration of Na+, K+ and ATPase activity – an enzyme important in the polymerization of amino acids, thus inhibiting the process of bonding the aminoacids to peptides and blocking DNA synthesis (Hordyjewska and Pasternak, 2004).

Long-term exposure to fluorine compounds induces also morphological changes in many organs, leading to an impairment of their function. Particularly sensitive to the effects of fluorides are the liver (Kołodziejczyk et al., 2000; Shashi and Thapar, 2000; Chinoy et al., 1993) and the kidneys (Ogilvie, 1953). Pathological changes occur also in the heart (Shashi and Thapar, 2001), pancreas and lungs (Stawiarska-Pięta et al., 2008, 2009). Numerous previous studies demonstrated that the disturbances in redox processes play important role in the pathomechanism of the observed changes.

Abbreviations: ALD, fructose-1,6-bisphosphate aldolase; CAT, catalase; GR, glutathione reductase; GSH-Px, glutathione peroxidase; GST, glutathione transferase; SOD, superoxide dismutase; LDL, lactate dehydrogenase; MDA, malondialdehyde; MDH, malate dehydrogenase; ROS, reactive oxygen species; SDH, sorbitol dehydrogenase; vit E, vitamin E.

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Fluoride ions display the ability to initiate a process called the respiratory burst and stimulate the production of superoxide radical (Chlubek, 2003; Rzeuski et al., 1998). Reactive oxygen impairs the cell function by interaction with its constituents, e.g. it changes the structure and permeability of cell membranes, by oxidizing the enzymes and other structural and transmembrane proteins (Bartosz, 2004). Numerous studies indicate the increased lipid peroxidation processes measured as malonodialdehyde (MDA) concentration. Increased concentration of MDA was demonstrated in the serum, liver and brain of animals exposed to the action of sodium fluoride (Shivarajashankara et al., 2001; Grucka-Mamczar et al., 2009). Moreover, fluorine affects the activity of enzymes constituting the cell antioxidant system (superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and transferase), which role is to protect against free radicals (Rzeuski et al., 1998; Chinoy, 2003; Shivarajashankara and Shivashankara, 2001). It can be suggested therefore that administration of antioxidants may prevent the oxidative stress associated with the exposure to fluoride by strengthening the antioxidant system of the organism.

The aim of this study was to assess the impact of co-administration of vitamin E and methionine on the activity of certain enzymes regulating carbohydrate metabolism and enzymes associated with glutathione as well as to examine the morphology of the liver in rats exposed to sodium fluoride.

2. Material and methods

The present experiment was conducted on 18 male Wistar rats with the initial body weight 393.75 ± 15.5 g. At the beginning of the experiment, the rats were 4 months old. The animals were obtained from the Experimental Centre of the Silesian Medical University in Katowice. Rats were divided into three groups of six animals: control group, in which animals received distilled water for drinking; the experimental group I (NaF) and the experimental group II (NaF + vitamin E + Met), in which rats were given 10 mg NaF/kg of body weight/24 h. In addition, animals in group II received 3 mg of vitamin E/per rat/24 h and 2 mg of methionine/per rat/24 h. We administered orally 4 mg NaF in 10 ml of aqueous solution to each rat per day. Vitamin E and methionine were administered orally with the LABOFEED. NaF used in the experiment was produced by POCH Gliwice Poland (sodium fluoride p.a.). The concentration of fluoride in fodder was 0.7 mg per kg of the feed. Animals were given distilled water.

During the experiment, animals were housed in separate cages. After administration of the test compounds rats received *ad libitum* access to distilled water and standard laboratory food (LABOFEED). The experiment lasted for 35 days. After this time the rats were anesthetized by intraperitoneal administration of 0.5 ml 1% hexobarbital/100 g of rat body weight. For pathological examination, the livers were dissected and fixed in formalin. Pathomorphological evaluation was conducted on preparations made by standard paraffin method, followed by staining with hematoxylin and eosin (Zawistowski, 1998). Preparations were examined using the light microscope (Olympus) under magnification 50×, 100×, 200×, 400×, 600×, and the microphotographs were taken with Olympus digital camera.

Moreover, in supernatants obtained after homogenization of rat liver slices, the activity of the following enzymes was assayed: fructose-1,6-biphosphate aldolase (ALD) (EC 4.2.1.7 and EC 4.1.2.13), malate dehydrogenase (MDH) (EC 1.1.37) and lactate dehydrogenase (LDH) (EC 1.1.1.27) using colorimetric methods, sorbitol dehydrogenase (SDH) (EC 1.1.1.21 and EC 1.1.1.14) by spectrophotometry (Krawczyński, 1972), the activity of glutathione peroxidase (GPx) (EC 1.11.1.9) using Paglia's method (Paglia and Valentine, 1967), glutathione transferase (GST) (EC 2.5.1.18) using kinetic method (Habig and Jacoby, 1981), and glutathione reductase (GR) (EC 1.6.4.2 and EC 1.8.1.7) using Richterich's method (Richterich, 1971).

Statistical analysis was performed with STATISTICA 9 Stat Soft, Inc. Mean (X) and SD values were used as the indicators of descriptive statistics. The data was checked for the normality of the distribution using the Shapiro–Wilk test. The specific comparisons between respective groups were made with Mann–Witney's U test. The criterion for statistical significance was set at $p < 0.05$.

All the testing procedures were approved by the Ethical Committee of the Silesian Medical University in Katowice.

3. Results

3.1. Histopathological evaluation

3.1.1. Macroscopic evaluation

No changes were detected in the shape, texture, color and size of the liver following both fluoride intoxication and the administration of sodium fluoride with methionine and vitamin E.

3.1.2. Microscopic evaluation

The representative microscope images of the intact rat liver observed in control group are shown in Fig. 1, while Figs. 2–6 show the morphological images obtained from experimental groups I (NaF) and II (NaF + vitamin E + Met).

Our study demonstrated regressive changes of the liver cells in animals exposed to sodium fluoride (experimental group I). Necrosis of single cells (Fig. 2), as well as a focal necrotic lesions covering larger areas of hepatic parenchyma (Fig. 3) were detected. These changes were accompanied by microinfiltrations in the stroma around triads and in the lobules among liver cells (Fig. 4), and also by the hyperemia (Fig. 5).

Administration of antioxidants (vitamin E and methionine) to rats exposed to sodium fluoride effectively attenuated the adverse effects evoked by exposure to sodium fluoride. The animals exhibited no regressive changes (Fig. 6), only occasional small clusters of mononuclear cells were found within the hepatic lobules.

3.2. Results of biochemical examinations

3.2.1. Activity of fructose-1,6-bisphosphate aldolase in rats' liver

Table 1 presents ALD activity in the livers of experimental animals. The statistically significant decrease was detected in the

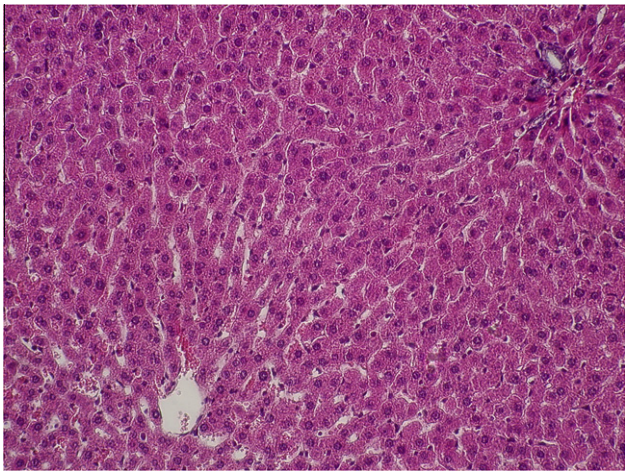

Fig. 1. Control group. Liver of rat. Normal pattern. H-E staining. 200×.

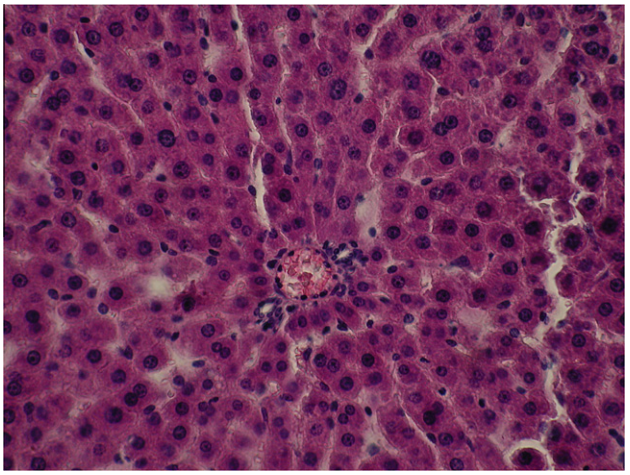

Fig. 2. Group I (NaF). Liver. Hepatocytes' diseminated necrosis. H-E staining. 400×.