INFLUENCE OF VITAMIN E ON LIVER MORPHOLOGY AND ACTIVITY OF CARBOHYDRATE ENZYMES OF RATS EXPOSED TO SODIUM FLUORIDE

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SUMMARY: The aim of this study was to examine the influence of vitamin E on the liver morphology and carbohydrate enzyme activities of rats intoxicated with sodium fluoride (NaF). Eighteen adult male Wistar rats on a standard rat diet were divided into three equal groups for a five-week experiment: a control group on distilled water; an experimental group I dosed with 4 mg NaF/rat/24hr in the drinking water; and a group II with the same dose of NaF plus 3 mg vitamin E/rat/24hr. Pathomorphological changes in the liver were assessed by paraffin histopathology, and changes in protein content and carbohydrate enzyme activities of aldolase, malate dehydrogenase, lactate dehydrogenase, and sorbitol dehydrogenase were determined by standard methods. Under conditions of this study with rats, supplemental vitamin E partially but did not completely prevent the toxic effects of NaF on the liver histopathology and carbohydrate enzyme activities.

Keywords: Carbohydrate enzymes; Fluoride and rat liver; Liver morphology; Vitamin E and rats.

INTRODUCTION

Exposure of animals to sodium fluoride (NaF) causes pathomorphological changes in many organs: kidneys,[1,2] lungs,[3] brain,[4,5] gonads,[6] and liver.[1,7] These toxic effects of fluoride (F) are related to its negative impact on metabolic processes such as carbohydrate, adipoid, and proteinaceous metabolism[8] by affecting the activity of enzymes and disturbing cellular homeostasis.[8]

For some time, extensive research has been conducted to determine the contribution of F to oxidative stress and pathogenic changes in animal organs,[9-10] in cells (*in vitro*),[11] and in people with endemic fluorosis.[12] Exposure to F induces increased lipid peroxidation measured as malondialdehyde (MDA)[11,12] by altering the activity of antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx).[13-14] As a result, disadvantageous results of radical-genesis are counteracted with an antioxidative barrier composed of antioxidative enzymes, and non-enzymatic antioxidants. Studies have also shown that oxidative stress can be prevented, or diminished by a protective barrier of an organism by a diet enriched with antioxidative vitamins (A, E, and C), or nonvitamin antioxidants (coenzyme Q10, methionine, or lipoate).[15] Advantageous activity of antioxidants has been observed in research on the fetotoxicity of F.[16] The aim of the present study was to evaluate the impact of vitamin E on the morphology and activity of carbohydrate enzymes of the liver of rats.

MATERIALS AND METHODS

The study was performed on eighteen 4-month-old Wistar male rats weighing 393.71±15.48 g. The animals, supplied by the Study Centre of the Medical

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University of Silesia in Katowice, were divided into 3 groups: a control group—kept on distilled drinking water; group I (NaF)—given NaF aqueous in doses of 4 mg NaF/rat/24hr; group II (NaF+vitamin E)—given NaF in doses of 4 mg NaF/rat/24hr in aqueous solution and vitamin E in the amount of 3.0 mg/rat/24hr (vitamin E 300 mg/mL, Medana Pharma Terpol, Sieradz, Poland). Four mg NaF was administered orally in 10 mL of aqueous solution per day to each rat. Vitamin E was administered orally with the standard ALTROMIN rat chow containing 0.7 mg F/kg. The concentration of vitamin E in the ALTROMIN diet was 75 mg/kg. The total dose of vitamin E (consumed from the food and administered additionally) was ca. 6 mg/rat/24hr (assuming that rats consumed ca. 40 g of food daily, corresponding to 3 mg of vitamin E per day from the diet.) This same dose of vitamin E was also consumed by the rats in the control group and group I.

The NaF used in the experiment was supplied from POCH Gliwice, Poland. The experiment lasted 5 weeks with *ad libitum* access of the rats to water and the standard ALTROMIN diet. At the end of 5 weeks, the rats were fatally anaesthetised by intraperitoneal injection of 0.5 mL of 1% hexobarbital/100 g of body mass. At necropsy, the liver (right lobe) was removed for histopathological examination, and liver fragments were prepared for biochemical examination.

Histopathology: After being preserved in formalin, the liver fragments were studied for morphological changes by the standard paraffin technique, stained with hematoxylin and eosin.[17] The microphotographs were taken with a use of a digital camera (CAMEDIA C-3040).

Biochemical analyses: Ten percent liver homogenates were prepared in 0.9% NaCl at 4ºC. The following data were determined in the liver supernatants: *a*) protein concentration by Lowry's method,[18] *b*) activity of carbohydrate change enzymes, malate dehydrogenase (MDH-EC 1.1.37), aldolase fructose-1,6-bisphosphate (ALD-EC 4.2.1.7), and lactate dehydrogenase (LDH-EC 1.1.1.27) by colorimetry, and *c*) sorbitol dehydrogenase (SDH-C 1.1.1.21) by spectotrophotometry.[19]

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

Statistical analysis: STATISTICA 9 Stat Soft Inc. STATISTICA program was used. For comparison of differences between the groups, the Dunnett's test was applied. Changes of $p<0.05$ were assumed to be statistically significant. Values located within $0.05<p<0.1$ were recognized to indicate a tendency toward a statistically significant difference.

RESULTS

Enzyme activity effects: Results of the biochemical study are presented in Tables 1–4. As seen in Table 1, a significant decrease in the activity of aldolase (ALD) occurred in the NaF group I rats compared to that of the control group. Administration of vitamin E together with NaF (group II) resulted in a statistically important rise of the activity of ALD compared to the activity in the NaF group I

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Table 1. Activity of aldolase in rat liver (IU/g protein)

Group of rats	Mean actvity	±SD	% compared with the control	% compared with the NaF group
Control	95.94	7.01	-	-
Group I (NaF)	76.60	15.03*	20.16 ↓	-
Group II (NaF+vitamin E)	96.54	16.76†	0.62 ↑	26.03 ↑

Compared with the control: *$p<0.05$; compared with the NaF group: †$p<0.05$.

On the other hand, as seen in Table 2, the activity of lactate dehydrogenase (LDH) showed a significant increase in the NaF group I, which was moderated by vitamin E in group II.

Table 2. Activity of lactate dehydrogenase in rat liver (IU/g protein)

Group of rats	Mean actvity	±SD	% compared with the control	% compared with the NaF group
Control	73.35	13.55	-	-
Group I (NaF)	84.82	2.61*	15.63 ↑	-
Group II (NaF+vitamin E)	76.78	9.2	4.67 ↑	9.48 ↓

Compared with the control: *$0.05<p<0.1$.

The activity of sorbitol dehydrogenase (SDH) also showed a significant increase in group I, which, however, was not moderated by vitamin E (Table 3).

Table 3. Activity of sorbitol dehydrogenase in rat liver (IU/g protein)

Group of rats	Mean actvity	±SD	% compared with the control	% compared with the NaF group
Control	3.88	0.75	-	-
Group I (NaF)	5.88	1.72*	51.50 ↑	-
Group II (NaF+vitamin E)	5.24	0.93	35.05 ↑	10.88 ↓

Compared with the control: *$p<0.05$

Finally, malate dehydrogenase (MDH) showed a non-significant increase in group I that was not appreciably altered with vitamin E (Table 4).

Table 4. Activity of malate dehydrogenase in rat liver (IU/g protein)

Group of rats	Mean actvity	±SD	% compared with the control	% compared with the NaF group
Control	271.05	9.73	-	-
Group I (NaF)	297.16	47.95	9.63 ↑	-
Group II (NaF+vitamin E)	294.15	28.16	8.52 ↑	1.01 ↓

Histopathological evaluation: No macroscopic changes in the shape, size, colour, and the consistency were observed in a typical liver of the rats in the control group I (Figure 1). Pathomorphological changes in the NaF group I included dispersed necrosis and congestion in lobules (Figure 2) and infiltrations from mononuclear cells in the vicinity of blood vessels (Figure 3). Necroses comprising larger fragments of lobule and vacuolar degeneracy were also observed in the liver of two rats in group I. As seen in Figure 4, administration of vitamin E in group II essentially stopped the modifications being formed in the liver. The congestion receded, and infiltrations were observed in 3 rats in group II.

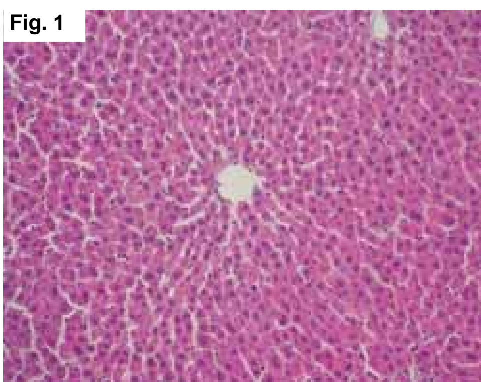

Figure 1. Control group. Liver. Normal pattern. H-E staining. 200×.

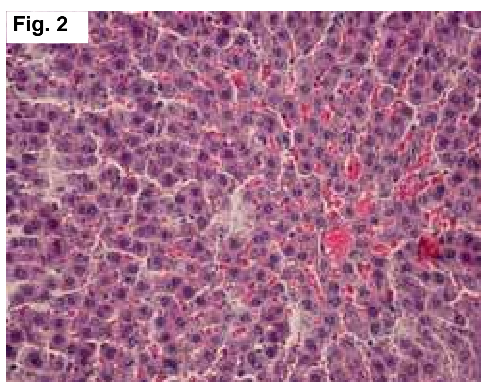

Figure 2. Group I (NaF). Liver. Hyperemia and hepatocytes necrosis spread. H-E staining. 400×.

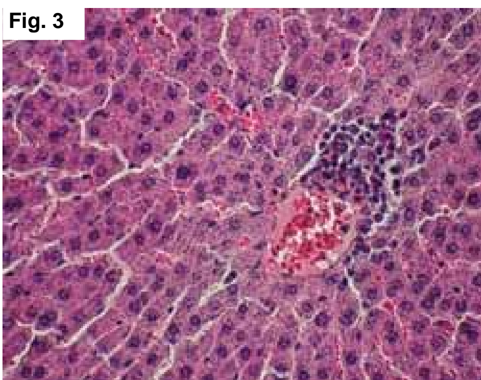

Figure 3. Group I (NaF). Liver. Infiltrations from mononuclear cells around the blood vessel and hyperemia. H-E staining. 400×.

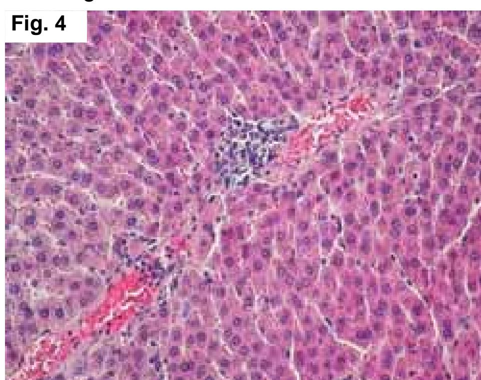

Figure 4. Group I (NaF + vitamin E). Liver. Infiltrations mononuclear cells around the blood vessel. H-E staining. 200×.

DISCUSSION

The observed decrease in fructose-1,6-bisphosphate aldolase (ALD) activity from exposure of the rats to NaF indicates suppression of the D-fructose–1,6-phosphate transformation into dihydroxyacetone phosphate. The consequence of this inhibition is a defect in monosaccharide (glucose, fructose, and galactose) metabolism and a decrease in the production of acetyl-CoA, which is indispensable to generate energy necessary for normal functioning of cells.[20] Similar results showing an inhibiting impact of NaF on the activity of ALD are on record.[21,22] Other studies show an increase from NaF in the activity of ALD in rat liver[22] or indicated a lack of NaF impact on ALD activity in the pancreas.[23]

Also observed in the present study was a statistically significant rise in the activity of sorbitol dehydrogenase (SDH). With involvement of $NADP_2$, this enzyme catalyses the conversion of fructose into D-sorbitol. A rise in the activity of this enzyme leads to an increase in glucose synthesis and stimulation of the pentose phosphate pathway.[20] In our previous research, both an increase[21-23] and a decrease[22,23] or a lack of changes in the activity of this enzyme[24] have been observed.

Here, no significant effects of NaF on the activity of malate dehydrogenase (MDH) were found. With the same dosage of NaF, we previously found a significant increase in the activity of MDH in the pancreas of rats.[23] On the other hand, Grucka-Mamczar et al.[25] observed a statistically significant decrease in MDH activity in the serum of rats exposed to 100 ppm F in their drinking water.

In contrast to negative results reported by Birkner et al.,[26] we found a significant increase in the activity of lactate dehydrogenase (LDH) in the liver of rats intoxicated by NaF in the drinking water. In a chronic study, Birkner et al.[26] also observed no significant change in SDH activity in the liver. The increase in the activity observed here of SDH and LDH—two enzymes usually inhibited by F ions—might be an adaptative response.

As found this study, supplemental administration of the well-known antioxidant vitamin E successfully prevented significant changes from NaF to the activity of ALD and LDH in the liver of the rats in group II. The activity in this group was similar to that in the control group. Vitamin E did not protect against the change in SDH activity after intoxication by NaF.

The changes in biochemical activity were accompanied by pathomorphological changes in the liver of rats intoxicated with F. The observed abnormalities of the liver, manifested as necrotic lesions of hepatocytes and infiltrations composed of mononuclear cells in the liver stroma indicate that NaF induces a toxic effect and an immune response. Hyperemia accompanying these changes is a component of the response to liver cell damage.

Although slight pathomorphological changes were observed in the livers of 3 rats, a similar, protective effect of vitamin E against such changes was demonstrated in the pancreases and lungs of male rats intoxicated with NaF.[23] Moreover, fully successful protective activity in the same organs was observed

when a combination of antioxidants including vitamins A and E and coenzyme Q were jointly administered to female rats.[3] Recently, we also reported that administration of vitamin E and methionine counteract changes in the morphological abnormalities in the liver induced by NaF.[27] In 2001, Chinoy and Patel[28] reported similar findings on the reversal of toxic effects of F on the ovary and uterus of mice by vitamin E. Afterward, Chinoy et al.[29] observed that vitamin E also helped to reverse the toxicity of NaF + As_2O_3 to the gastrocnemius muscle of mice. On the other hand, in a much earlier preliminary report, Burgstahler[30] found that supplemental vitamin E gave only marginal protection against dental fluorosis in rats. In his experiments the rat diet already contained the recommended level of vitamin E. However, he observed that the development of dental fluorosis in the vitamin E-supplemented rats, especially in the females, was less pronounced.

To sum up, the observed preventive effect of vitamin E (α-tocopherol) results from the fact that it is appears to be the most potent fat-soluble, membrane-renewable antioxidant. Several studies have shown that vitamin E is responsible for protection of an organism against harmful activity of an excessive quantity of free radicals.[15,28,31] It apparently stabilizes cellular and subcellular membranes by means of Van der Waals' interactions with lipid chains of adipose acid residues. Thus, vitamin E influences the activity of enzymes such as protein kinase C and phospholipase A2.[32,33] The suppression of these enzymes in inflammatory cells (monocytes) substantially diminishes the production of free radicals and their effects. As a non-enzymatic antioxidant nutrient, vitamin E is a natural prerequisite for prevention of adverse changes caused by exposure to F.

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