LIPID BALANCE IN RATS WITH FLUORIDE-INDUCED HYPERGLYCEMIA

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SUMMARY: Fourteen-week-old male Wistar FL strain rats were administered sodium fluoride in a single intraperitoneal dose (35 mg NaF/kg bw) and after 90 min were fatally anesthetized. In a separate experiment, four-week-old male rats of the same strain were given fluoride in their drinking water (50 or 100 mg F^-/L) for four months and then anesthetized. Both experiments produced hyperglycemia, accompanied by a statistically significant increase in the concentrations of fluoride in the blood serum. Hypertriacylglycerolemia also occurred in the long-term intoxication experiment, thereby indicating disturbances of lipid metabolism under the influence of NaF, which are similar to those observed in diabetes mellitus or in starvation.

Keywords: Acute fluoride intoxication; Chronic fluoride intoxication; Hyperglycemia in rats; Serum cholesterol; Serum fluoride; Serum glucose; Serum triacylglycerols; Sodium fluoride.

INTRODUCTION

In recent years there has been considerable interest in fluoride-induced hyperglycemia. So far, how it occurs has not been clearly determined. In 1993, Shashi[1] reported an experiment carried out on albino rabbits, which were administered increasing doses of sodium fluoride for four months. Changes occurred in the biosynthesis of glycogen in the pancreas, as well as necrosis of pancreatic cells. A few years later, Vinals *et al*[2] discovered that fluoride inhibits the activity of tyrosine kinase, an insulin receptor which they purified from skeletal muscles of rats and human placenta. On the other hand, nearly 25 years ago, McGown and Suttie[3] presented experimental evidence that fluoride induces the release of epinephrine in rats, thereby resulting in hyperglycemia. Hyperglycemia after intoxication of fluoride was also reported by Sakurai *et al* [4] and by Appleton.[5]

The purpose of the present study was to induce hyperglycemia in rats administered sodium fluoride in an acute and a prolonged manner and to examine the resulting lipid balance. Assessment of the latter was based on measurement of the concentration of cholesterol and triacylglycerols in serum, which are synthesised in the liver, an organ that, besides the kidneys, is most exposed to the action of ingested fluoride.[6]

MATERIALS AND METHODS

Two separate experiments were conducted on male rats of the Wistar FL strain obtained from the Central Animal Farm Breeding Experimental Animals of the Medical University of Silesia in Katowice.

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In the acute version of the experiment, nine 14-week-old male rats having an average body mass of 203 ± 15 g were administered 0.5 mL of aqueous sodium fluoride intraperitoneally in a dose of 35 mg NaF/kg of body mass. The control group of ten rats with an average body mass of 201 ± 20 g were administered the same volume of isotonic salt solution intraperitoneally. After 90 min, all the rats were fatally anesthetized with ether and blood was taken from their hearts.

In the long-term experiment, thirty 4-week-old male rats randomly divided into three groups of 10 animals each and given distilled water *ad libitum* for three weeks. After completion of this adaptation period, two of these groups of rats were given water containing NaF. The first group received water containing 50 mg F^-/L (2.63 mmol/L), and the second group received water containing 100 mg F^-/L (5.26 mmol/L). The third group served as controls and continued to receive distilled water.

All the animals were maintained on a 12-hr light/dark cycle and were fed *at libitum* with granulated fodder Altromin (Germany) analyzing for 0.7 mg F^-/kg. After four months, these rats were killed by etherisation, and blood was taken from their hearts (per Agreement of Bioethical Committee of the Silesian Medical School in Katowice No NN-043-101/96, dated 04.06.1996 referring to the use of animal models in the studies of the patho-mechanism of diabetes in rats).

The concentrations of glucose, fluoride, cholesterol, and triacylglycerols in the blood serum were determined as follows: glucose was measured by the CORMAY Kit (Poland); fluoride was determined by a fluoride ion selective electrode (ORION 96-09, USA in 1.0 mL of serum with 1.0 mL of TISAB buffer at pH 5.0; cholesterol was estimated using the kit manufactured by POINTE SCIENTIFIC (USA, cat. No. C 7509-400); and triacylglycerols were measured with a kit also provided by POINTE SCIENTIFIC (USA, cat. No. T 7531).

Statistical analysis was performed using Statistic 6.0 PL software. Statistical methods included calculation of means, standard deviation (SD), and standard error of the mean (SEM). Shapiro-Wilk's W test was used to check the normalcy and Levene's test to check the homogeneity of variances. An analysis of variance or Kruskal-Wallis ANOVA test was used for multiple comparisons of data. Additional statistical comparisons were made by Student's t test, with separate variance estimates, or the Mann-Whitney U test. A value of $p<0.05$ was considered to be of significance.

RESULTS

Results of the acute version of the experiment, in which rats were administered intraperitoneally a single dose of 35 mg NaF/kg body mass, are shown in Table 1. An almost 40-fold increase in the concentration of fluoride in the blood serum and a simultaneous 47% increase in the concentration of glucose occurred. On the other hand, the serum concentration of cholesterol and triacylglycerols in these animals was unchanged compared to the control group

Table 1. Concentration of fluoride, glucose, cholesterol, and triacylglycerols in blood serum of rats receiving a single intraperitoneal injection of 35 mg of NaF/kg bw and in the control group

Concentration in serum	Control group mean ± SD	Study group mean ± SD	p value
Fluoride mol/L	4.4 ± 0.20	177.0 ± 15.0	<0.001
Glucose mmol/L	3.18 ± 0.72	4.67 ± 0.77	<0.001
Cholesterol mmol/L	2.72 ± 0.17	2.59 ± 0.16	0.098
Triacylglycerols mmol/L	0.48 ± 0.09	0.44 ± 0.10	0.108

In the long-term version of the experiment, in which rats were administered 50 and 100 mg of F^-/L in their drinking water, we found a statistically significant 12% reduction of body weight in the group receiving the higher concentration (Table 2).

Table 2. Mean body weight (g) of control rats and rats receiving 50 and 100 mg of F^-/L in their drinking water for four months

	Before study mean ± SD	p value	After 4 months mean ± SD	p value
Control	114.9 ± 10.78		335.0 ± 23.24	
50 mg F^-/L	107.6 ± 9.40	0.124	327.1 ± 32.10	0.455
100 mg F^-/L	110.2 ± 9.63	0.317	294.7 ± 44.44	0.020

As can be calculated from the data in Table 3, the group administered 50 mg of F^-/L had a highly significant 248% increase in the serum concentration of fluoride, and in the group administered 100 mg of F^-/L an even greater increase of 532%. At the same time, there was also a statistically significant 63% and 82% increase in the serum concentration of glucose in the two groups, respectively. Although the concentration of cholesterol in the blood serum did not change, the serum concentration of triacylglycerols in the rats receiving 100 mg of F^-/L in their drinking water for four months showed a 30% statistically significant increase compared with the control group.

Table 3. Concentration of fluoride, glucose, cholesterol, and triacylglycerols in blood serum of rats receiving F^- for 4 months in their drinking water and in the control group

Concentration in serum	Control group Mean ± SD	50 mg F^-/L group Mean ± SD	100 mg F^-/L group Mean ± SD	ANOVA p value
Fluoride µmol/L	3.59 ± 0.71	12.5 ± 1.47	22.7 ± 4.79	<0.001
Glucose mmol/L	3.13 ± 0.71	5.10 ± 1.42	5.69 ± 1.14	<0.001
Cholesterol mmol/L	1.84 ± 0.28	1.71 ± 0.22	1.64 ± 0.23	0.187
Triacylglycerols mmol/L	1.04 ± 0.23	0.94 ± 0.19	1.35 ± 0.24	<0.001

DISCUSSION

After ingestion of food, fatty acids — both those synthesised *de novo* from excess glucose and those provided to the liver from residual chylomicrons or particles of the LDL fraction — are usually utilized for the synthesis of triacylglycerols. Some of these acids are used for the production of phospholipids and the esterification of cholesterol. Besides degradation of residual chylomicrons, liver plays a vital role in the metabolism of cholesterol. Although nearly all of the cells of body tissues contain enzymes necessary for the synthesis of cholesterol, that process takes place most rapidly in the liver, which produces 85% of the body's cholesterol.[7]

In our study we assessed the concentrations of both triacylglycerols and cholesterol produced by the liver, which are a good index of lipid metabolism in the body, especially since the liver, as mentioned above, is the organ most exposed to the action of xenobiotics, including fluoride. Although no profound changes in the concentration of serum cholesterol and triacylglycerols were seen in the acute phase of the experiment, which lasted only 90 minutes, we observed a significant 30% increase in the concentration of triacylglycerols in the blood serum after prolonged administration of sodium fluoride in the dose of 100 mg of F^-/L of drinking water (Table 3).

In studies with male albino rabbits administered sodium fluoride in doses of 5, 10, 20, and 50 mg/kg bw/day, Shashi[8] found hyperphospholipidemia, Hypertriacylglycerolemia, and hypercholesterolemia in testes being indicative of increased biosynthesis of lipids in response to the toxic action of fluoride. In our study, the concentration of serum cholesterol in the group of male rats undergoing prolonged administration of sodium fluoride did not undergo significant changes.

Fluoride induced hyperglycemia in both experiments. It seems to be a result of increased hepatic glycogenolysis.[9] Fluoride increases cAMP level thereby activating cAMP-dependent protein kinase, which is responsible for activation of glycogen phosphorylase in the liver and triacylglycerol lipase in the adipose tissue. Therefore, a higher rate of triacylglycerol synthesis resulting in hypertriacylglycerolemia could be caused by increased lipolysis and fatty acid release from the adipose tissue. This, we believe, may be due to a generally negative influence of sodium fluoride upon metabolism, as shown in our previous studies,[10,11] as well as a direct influence of fluoride upon the adipose tissue as an endocrine organ.

The endocrine role of adipose tissue is best characterised by the anti-obesity hormone leptin, discovered and identified in 1994. Total deficit of leptin or insensibility to leptin is caused by diabetes, among other conditions.[12] Normally, leptin has an inhibiting influence on acetyl-CoA carboxylase. Inhibiting the activity of that enzyme leads, via reduction of the activity of malonyl-CoA, to blocking the synthesis of fatty acids and lowering the concentration of triacylglycerols.[13] Perhaps in fluoride-induced hyperglycemia, deficiency of leptin or resistance to leptin causes increased synthesis of triacylglycerols.

It is also conceivable that sodium fluoride, by inhibiting the activity of the insulin receptor,[2] also inhibits the activity of the leptin receptor, since the expression of the leptin receptor gene is most conspicuous in the β cells of the pancreas.[14,15] Should the ability of β cells of the pancreas be impaired in rats with hyperglycemia induced by the higher concentration of fluoride in the drinking water, a defective leptin receptor could result. In this connection, it is interesting that the leptin receptor (OB-R) mutation in mice with diabetes results from defective STAT (Signal Transducer and Activator Transcription) signalling by the leptin receptor.[14]

In our study we also found a statistically significant increase in the concentration of fluoride in rat blood serum in both the acute and the long-term version of the experiment (Tables 1 and 3). This, however, is a direct result of the fact that NaF is almost completely absorbed by the intestine. It is well confirmed that increased fluoride concentration in serum is dependent on the dose of F^-.[16-18]

As found here, prolonged administration of sodium fluoride to rats at 100 mg F^-/L in their drinking water causes disturbances in their lipid balance, which finds expression in hypertriacylglycerolemia, while the explanation for how it occurs calls for further studies, *e.g.*, assessment of leptin concentration in the serum of these animals.

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Published by the International Society for Fluoride Research
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