62   Research report   Influence of NaF and caffeine on carbohydrate metabolism   62
Fluoride 40(1)62–66   in rat serum and liver
January-March 2007   Grucka-Mamczar, Birkner, Zalejska-Fiolka, Machoy, Kasperczyk, Błaszczyk

INFLUENCE OF EXTENDED EXPOSURE TO SODIUM FLUORIDE AND CAFFEINE ON THE ACTIVITY OF CARBOHYDRATE METABOLISM ENZYMES IN RAT BLOOD SERUM AND LIVER

Ewa Grucka-Mamczar,[a] Ewa Birkner,[a] Jolanta Zalejska-Fiolka,[a] Zygmunt Machoy,[b]
Sławomir Kasperczyk,[a] Iwona Błaszczyk[a]

Szczecin, Poland

SUMMARY: A 50-day study of the influence of fluoride (F) and caffeine on carbohydrate metabolism enzymes was conducted on 18 male Sprague-Dawley rats, 4.5 months old, housed in separate cages at 22–25°C on a 12-hr light/dark cycle. The rats were divided into three groups with six rats in each group: control, F, and F+caffeine. F was administered by intubation in water as sodium fluoride (NaF) at a dosage of 4.9 mg F ion/kg bw/day and at the same dosage plus 3 mg caffeine/kg bw/day. After 50 days, the concentration of F in the blood serum was approximately double that of the control group, and the blood glucose increased marginally in the F and F+caffeine groups by 12% and 18%, respectively. In the liver, the activity of sorbitol dehydrogenase (SDH) decreased significantly in the F group by 45% but increased non-significantly in the F+caffeine group by 13%. Also in the liver, the activities of aldolase (ALD) and lactate dehydrogenase (LDH) in the F group increased by 13% and 16%, respectively, but in the F+caffeine group they decreased by 16% and 4%. In the serum, the activity of SDH increased very significantly in the F and the F+caffeine groups by 212% and 186%, respectively. On the other hand, the activities of ALD and LDH in the F group decreased by 15% and 52%, respectively, whereas in the F+caffeine group the activity of ALD increased by 17% and the activity of LDH decreased by 70%. CONCLUSION: In the liver, fluoride (F) ions inhibited the transformation of sorbitol into fructose, whereas caffeine promoted this conversion. Glycolysis in the liver, under the influence of F, did not appreciably change, whereas in the presence of caffeine it was slightly inhibited. Glycolysis in extra-hepatic tissues (serum), under the influence of F, was slightly inhibited; however, it was markedly intensified by caffeine. Overall, a more profound influence by caffeine on carbohydrate enzyme activity was observed in blood serum (extra-hepatic tissues) than in the liver.

Keywords: Caffeine; Carbohydrate metabolism; Glycolysis in liver; Glycolysis in serum; Rat liver; Rat serum; Sodium fluoride in rats.

INTRODUCTION

In vitro studies indicate that fluoride (F) impedes the synthesis of DNA and protein, inhibits cell proliferation, and is cytotoxic in sufficiently high doses. Toxic effects of F in animals and humans may be attributable to one or more of these metabolic and biochemical effects.[1]

Caffeine is a common biologically active food component of the modern diet with potential health implications. In recent years, the food industry has introduced nontraditional sources of caffeine, including it in energy drinks, chewing gum, bottled water, and alcoholic beverages, all of which may make a significant contribution to the overall intake of caffeine in the general population.[2]

[a]For correspondence: Dr Jolanta Zalejska-Fiolka, Department of Biochemistry, Silesian Medical University, 41-808 Zabrze, 19 Jordana Str, Poland: E-mail: jfiolka@slam.katowice.pl; [b]Department of Biochemistry and Chemistry, Pomeranian Medical University, Szczecin, Poland.

63   Research report
       Fluoride 40(1)62–66
       January-March 2007

Influence of NaF and caffeine on carbohydrate metabolism   63
in rat serum and liver
Grucka-Mamczar, Birkner, Zalejska-Fiolka, Machoy, Kasperczyk, Błaszcyk

Various effects of F on enzymes associated with carbohydrate metabolism (including glycolysis) in experimental animals are well recognized. F is also known to induce hyperglycemia,[3] but little is known about the behaviour of these enzymes in the course of simultaneous administration of F and caffeine.

The purpose of this study was to examine the activity of the glycolysis enzymes aldolase (ALD), lactate dehydrogenase (LDH), and sorbitol dehydrogenase (SDH) in the blood serum and liver of rats after administration of NaF and caffeine.

MATERIALS AND METHODS

The study was carried out on 18 male Sprague-Dawley rats, 4.5 months old, which were obtained from the Central Farm of Experimental Animals of the Silesian University of Medicine in Katowice, Poland. The rats were divided into three groups of six animals each and were kept in cages individually at 22–25°C. A normal 12-hr day-night cycle was maintained, and there was unrestricted access to standard rat chow and distilled water. Group I was the control group; group II received 4.9 mg F from NaF/kg bw/day, administered in 10 mL of water by oral intubation; and group III received the same amount of F as group II plus 3 mg caffeine/kg bw/day in 10 mL of water by intubation. After 50 days, the rats were anesthetized with 30 mg thiopental/rat. The following parameters were determined in the blood serum:

1   the concentration of F ions by means of a F ion-selective electrode Orion 96-09 (USA),[4]

2   the concentration of glucose by Biochemtest Kit (POCH Gliwice, Poland, Cat. No. 178163149),[4] and

3   the activity of sorbitol dehydrogenase (SDH) (EC 1.1.1.21), aldolase (ALD) (EC 4.2.1.7), and lactate dehydrogenase (LDH) (EC 1.1.1.27) by Krawczyński methods.[5]

The activity of SDH, LDH, and ALD in the liver was determined in 10% w/v homogenates of that organ by the same methods.

Numerical results were subjected to statistical analysis using the U Mann-Whitney test.

RESULTS

Results of the experiments are presented in Tables 1, 2, and 3. Table 1 shows that the blood serum concentration of F ions in group II (F) and in group III (F+caffeine) approximately doubled (statistically significant) in comparison with the control group I. The concentration of glucose in group II increased by 12%, whereas in group III it increased by 18% (not statistically significant).

Table 1. Concentrations of F and glucose in rat serum

Rat group (n = 6/group)	Concentrations					
	F (ppm)			Glucose (mmol/L)		
	Mean	± SD	p-value	Mean	± SD	p-value
Control (I)	0.1	0.02	-	3.2	0.81	-
F (II)	0.2	0.04	0.004	3.6	0.60	0.262
F+caffeine (III)	0.2	0.03	0.004	3.8	0.62	0.200

64   Research report   Influence of NaF and caffeine on carbohydrate metabolism   64
        Fluoride 40(1)62–66   in rat serum and liver
        January-March 2007   Grucka-Mamczar, Birkner, Zalejska-Fiolka, Machoy, Kasperczyk, Błaszcyk

Table 2 shows that the activity of SDH in the liver of the F group II decreased significantly by 45%, but it increased in the F+caffeine group III by 13% (not significant). The activity of ALD in the liver of the F group increased by 13%, whereas in the F+caffeine group it decreased by 16% (neither statistically significant). The activity of LDH increased non-significantly by 16% in the F group and decreased by only 4% in the F+caffeine group.

Table 2. Activities of SDH, ALD, and LDH in rat liver

Rat group (n = 6/group)	Activities								
	SDH (IU/g protein)			ALD (IU/mg protein)			LDH (IU/mg protein)		
	Mean	± SD	p-value	Mean	± SD	p-value	Mean	± SD	p-value
Control (I)	7.5	2.72	-	8.61	0.85	-	2.5	0.47	-
F (II)	4.1	2.10	0.037	9.73	1.38	0.109	2.9	0.47	0.109
F+caffeine (III)	8.5	5.24	0.873	7.19	1.79	0.078	2.4	0.46	0.873

Table 3 shows that the activity of SDH in blood serum increased very significantly by 212% in the F group and by 186% in the F+caffeine group. On the other hand, the activity of ALD in blood serum of the F group decreased non-significantly by only 15% and increased in the F+caffeine group by 17%. The activity of LDH in blood serum decreased significantly by 52% in the F group and in the F+caffeine group by 70%.

Table 3. Activites of SDH, ALD, and LDH in rat serum

Rat group (n = 6/group)	Activities								
	SDH (IU/L)			ALD (IU/L)			LDH (IU/L)		
	Mean	± SD	p-value	Mean	± SD	p-value	Mean	± SD	p-value
Control (I)	5.8	2.35	-	31.1	2.79	-	259.4	105.66	-
F (II)	18.1	3.83	0.004	26.5	6.63	0.336	123.9	66.61	0.025
F+caffeine (III)	16.6	6.68	0.010	36.3	2.00	0.004*	78.3	39.24	0.006

DISCUSSION

F in blood serum: In this study we applied doses of F and caffeine in accordance with the experiments reported by Chen and Whitford.[6] They reported that their results from recent acute studies with rats indicated that plasma F concentrations measured 4 hr after oral administration of F were considerably higher when the dose was given in coffee or in an aqueous solution of caffeine compared with when it was administered in water without caffeine.[7]

In our work we found the same concentration of F in blood serum of rats exposed to F as in those exposed to F+caffeine. The explanation for this difference between our results and those of the Whitford group just cited is not clear, but conceivably it might be due to factors such as differences in the strain of rats, time, diet, and how the F was determined in the blood serum.[7] Whitford also reported that when F was administered in coffee containing caffeine, but not in a caffeine solution or decaffeinated coffee, the concentration of plasma F was slightly higher the first 1 or 2 hr but not thereafter. The explanation for the difference between these findings and the earlier ones[6] is not known.[8]

65   Research report   Influence of NaF and caffeine on carbohydrate metabolism   65
        Fluoride 40(1)62–66   in rat serum and liver
        January-March 2007   Grucka-Mamczar, Birkner, Zalejska-Fiolka, Machoy, Kasperczyk, Błaszczyk

Sorbitol dehydrogenase (SDH) in blood serum and liver: In the liver, ovaries, sperm, and seminal vesicle cells there is a sorbitol dehydrogenase that can oxidize the sorbitol to fructose. The pathway from sorbitol to fructose in the liver provides a mechanism by which dietary sorbitol is converted into a substrate that can enter glycolysis or gluconeogenesis.[9] Our results show that F ions inhibit the activity of SDH in the liver but increase it in blood serum. Apparently F enhances the permeability of hepatocyte membranes (or the membranes of extra-hepatic cells), and the escape of SDH to blood follows suit. A similar dependence between the activity of SDH in blood serum and liver was noted in rabbits, in which experimental atherosclerosis was provoked, by adding 2.0 g% of cholesterol to food and adding NaF to drinking water (3 mg F/kg bw/24 hr).[10] These results may be related to the almost doubled increase in the concentration of F ions in blood serum of the experimental rats. Machoy found that when the level of F in blood and urine becomes double that of controls groups, a crucial interaction between F and numerous enzymes occurs in body fluids with respect to cell membranes.[11] Our studies indicate that F+caffeine increases SDH activity in hepatocytes, whereas F alone decreases it. Because glycolysis in the presence of F+caffeine can be inhibited, the sorbitol pathway delivers fructose to gluconeogenesis via phosphodihydroxyacetone and can increase the level of blood glucose. In agreement with this interpretation, Kessabi et al. reported that increased sorbitol dehydrogenase activity in serum was significant in sheep in the Darmous (endemic fluorosis) area of Morocco.[12]

Lactate dehydrogenase (LDH) in blood serum and liver: Although our study showed that neither F nor F+caffeine appreciably affected LDH activity in the liver, caffeine intensified the inhibitory effect of F on the activity of this enzyme in the blood serum, perhaps by a direct effect of F and F+caffeine on extra hepatic cells. Zakrzewska et al. observed that the LDH activity in sperm samples with the three lower concentrations of fluoride (20, 100 and 200 µmol/L) decreased nearly nine-fold.[13] Bogin et al. reported declines in LDH levels in the liver, kidney, heart, and skeletal muscles of mice drinking water containing 100 ppm NaF.[14] Similarly, Singh found that the activity of LDH in liver of mice given drinking water containing 25, 500, 100, and 200 ppm F for 16 weeks significantly decreased.[15] In our study, the clear inhibition of LDH activity in serum in the F+caffeine group demonstrates inhibition of glycolysis in extra-hepatic tissues like muscle and erythrocytes.

Aldolase (ALD) in blood serum and liver: The activity of ALD in liver under the influence of F+caffeine in liver seems to be inhibited (Table 2). This is especially interesting, since in the presence of F without caffeine, glycolysis in liver appears to be normal. It is known that caffeine is an inhibitor of phosphodiesterase and also causes an increase in cAMP and blood glucose.[16] Glucagon via an elevation cAMP level stimulates the synthesis of glucose and inhibits glycolysis in the liver,[16] which we found to be true here. On the other hand, in extra-hepatic cells, glycolysis is probably intensified because the activity of ALD in blood serum of rats in the F+caffeine group increased significantly in comparison with the control group, suggesting enhanced transport of glucose to extra-hepatic tissues under the

66   Research report   Influence of NaF and caffeine on carbohydrate metabolism   66
         Fluoride 40(1)62–66   in rat serum and liver
         January-March 2007   Grucka-Mamczar, Birkner, Zalejska-Fiolka, Machoy, Kasperczyk, Błaszczyk

influence of caffeine. This activation of glycolysis by F and caffeine in extrahepatic tissues can also be seen in our similar earlier findings with ALD activity in kidneys of rats exposed to the same concentrations of F and caffeine.[17] Under the influence of F in the absence of caffeine, the activity of ALD diminishes in blood serum (results without statistical significance), so that glycolysis in extra-hepatic tissues is inhibited.

CONCLUSIONS

1   Fluoride inhibited and caffeine activated transformation of sorbitol into fructose in the liver.

2   Glycolysis was inhibited by fluoride and intensified by caffeine in serum.

3   A more profound influence by caffeine on carbohydrate enzyme activity was observed in blood serum than in the liver.

ACKNOWLEDGMENTS

REFERENCES

1   Environmental Health Criteria 227, Fluorides, World Health Organization, Geneva 2002.
2   Robinson LE, Savani S, Battram DS, Mc Lare DH, Sathasivam P, Graham TE. Caffeine ingestion before an oral glucose tolerance test impairs blood glucose management in men with type 2 diabetes. J Nutr 2004;134(10):2528-33.
3   Grucka-Mamczar E, Birkner E, Kasperczyk S, Kasperczyk A, Chlubek D, Szamujło D, et al. Lipid balance in rats with fluoride-induced hyperglycemia, Fluoride 2004;37(3):195-200.
4   Birkner E, Grucka-Mamczar E, Zalejska-Fiolka J, Chlubek D, Kasperczyk S, Stawiarska-Pięta B, et al. Influence of sodium fluoride and caffeine on the concentration of fluoride ions, glucose and urea in blood serum and activity of protein metabolism enzymes in rat liver. Biol Trace Elem Res 2006;112:169-74.
5   Krawczyński J, editor. Diagnostyka enzymologiczna w medycynie praktycznej [Enzymologic diagnostics in practical medicine]. Warsaw: PZWL; 1972.
6   Chen X, Whitford GM. Lack of significant effect of coffee and caffeine on fluoride metabolism in rats. J Dent Res 1994;73(6):1173-9.
7   Chan JT, Qui CC, Whitford GM, Wheatherred JG. Influence of coffee on fluoride metabolism in rats. Proc Soc Exp Biol Med 1990;194(1):43-7
8   Whitford GM. The metabolism and toxicity of fluoride. 2nd revised ed. Basel: Karger; 1996.
9   Champe PC, Harvey RA. Lippincott's illustrated reviews: biochemistry 2nd ed. Philadelphia: J B Lippincott; 1994.
10 Birkner E, Grucka-Mamczar E, Kasperczyk S, Kasperczyk A, Zalejska-Fiolka J, Birkner B, et al. Effect of sodium fluoride on the activity of sorbitol dehydrogenase in plasma, and liver of rabbits with experimental atherosclerosis. Ann Acad Med Stetinensis 2004; 50:14-8.
11 Machoy Z. Biochemiczne mechanizmy działania związków fluoru [Biochemical mechanisms of fluorine compounds action]. F Med Cracov 1987;28:61-80.
12 Kessabi M, Boudarine JP, Braun JP, Lamnaouer D. Serum biochemical effects of fluoride in sheep of the Darmous area. Fluoride 1983;16(4):214-9.
13 Zakrzewska H, Udała J, Błaszczyk B. In vitro influence of sodium fluoride on ram semen quality and enzyme activities. Fluoride 2002; 35(3):153-60.
14 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.
15 Singh M. Biochemical and cytochemical alterations in liver and kidney following experimental fluorosis. Fluoride 1984; 17(2):81-93.
16 Baynes J, Dominiczak MH. Medical Biochemistry, Mosby 1999.
17 Birkner E, Grucka-Mamczar E, Żwirska-Korczala K, Zalejska-Fiolka J, Stawiarska-Pięta B, Kasperczyk S, et al. Influence of sodium fluoride and caffeine on the kidney function and free-radical processes in that organ in adult rats. Biol Trace Elem Res 2006;109:35-47.