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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

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ABSTRACT

The aim of the study was examining the effect of fluoride ions and caffeine administration on glucose and urea concentration in blood serum and the activity of protein metabolism enzymes and selected enzymes of the urea cycle in rat liver. The study was carried out using 18 male Sprague–Daowley rats (4.5 mo old). Rats were divided into three groups. Group I received distilled water ad libitum. Group II received 4.9 mg F^-/kg body mass/d of sodium fluoride in the water, and group III received sodium fluoride (in the above-mentioned dose) and 3 mg/kg body mass/d of caffeine in the water. After 50 d, the rats were anesthetized with thiopental and fluoride ions, glucose, and urea concentration in blood serum were determined. Also determined were the activities of aspartate aminotransferase, alanine aminotransferase glutamate dehydrogenase, ornithine carbamoylotransferase and arginase in liver homogenates. Liver was taken for pathomorphological examinations. The applied doses of F^- (4.9 mg/kg body mass/d) and F^- + caffeine (4.9 mg F^-/kg body mass/d + 3 mg caffeine/kg

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body mass/d) resulted in a statistically significant increase of fluoride ion concentration in blood serum, a slight increase of the glucose concentration, and no changes in the concentration of urea in blood serum. This might testify to the absence of kidney lesions for the applied concentrations of F^-. No change in the functioning of hepatocytes was observed; however, slight disturbances have been noted in the functioning of the liver, connected with the activation of urea cycle, increase of arginase activity, and accumulation of F^- in this organ. There was no observed significant influence of caffeine supplementation on the obtained results.

Index Entries: Fluoride ion; caffeine; rat; enzymes of urea cycles; protein metabolism enzymes.

INTRODUCTION

Most products of food digestion taken up by intestine pass through the liver. Liver degrades excessive amino acids, which remain unused after a meal consisting of carbohydrates. That organ also synthesises and releases ketone compounds and participates in the synthesis of cholesterol. The liver performs detoxification activities. One of the first activities of that type is the removal of ammonia split off of amino acids and its transformation into particles of urea in the Krebs urea cycle. Detoxification, however, applies to various toxins and xenobiotics *(1)*. Sodium fluoride can also be included among xenobiotics.

Fluoride ions find their way to the organism from the outside (tap and potable water); they are acumulated not only in hard tissues but also in soft ones (liver, kidneys) *(2–4)*, and recent studies revealed that caffeine increased the concentration of fluoride ions in blood plasma. In 1994, Chen and Whitford *(5)* discovered that after 2 h the administration of F^- in caffeine solution resulted in a higher concentration of fluoride ions in the plasma than the concentration of fluoride ions administered in water.

Taking into account the above observations as well as the substantial availability and use of caffeine (coffee, cola), we decided to examine the concentration of fluoride ions, glucose, and urea in blood serum and to assess the activity of protein metabolism enzymes (aspartate aminotransferase [AST], alanine aminotransferase [ALT], and glutamate dehydrogenase [GLDH]) and selected enzymes of the urea cycle (ornithine carbamoylotransferase [OCT-ase] and arginase) determined in rat liver after the administration F^- and F^- with caffeine in drinking water.

MATERIALS AND METHODS

The study was carried out using 18 male Sprague–Dawley 18 rats (4.5 mo old). The animals came from the Central Farm of Experimental Animals of the Silesian University of Medicine in Katowice, Poland.

Rats were divided into three groups of six animals. A normal day–night cycle was maintained for the rats; they also had unrestricted access to stan-

Table 1
Concentration of Fluoride Ions, Glucose, and Urea in Rat Blood Serum

Groups	Concentration								
	F^- [ppm]			glucose[mmol/L]			urea[mmol/L]		
	X	SD	p	X	SD	p	X	SD	p
group I	0.14	0.02	-	3.22	0.81	-	7.25	1.58	-
group II	0.21	0.04	0.004	3.61	0.60	0.262	7.39	0.54	0.575
group III	0.21	0.03	0.004	3.78	0.62	0.200	6.96	0.90	1.000

dard fodder. Group I was the control group and received distilled water ad libitum. Group II received 4.9 mg F^-/kg body mass/d of sodium fluoride in the water, and group III received sodium fluoride (in the above-mentioned dose) and 3 mg/kg body mass/d of caffeine in the water. After 50 d, the rats were anesthetized with thiopental at a dose of 30 mg/rat.

The following parameters were determined in the blood serum: the concentration of fluoride ions by means of ion-selective electrode Orion 96-09 (USA); the concentration of glucose by Biochemtest kit (POCh Gliwice, Poland; cat. no. 178 163 149); the concentration of urea by Biochemtest kit (POCh Gliwice, Poland; cat. no. 178 226 141). Liver homogenates (10% w/v) were made and the following activities were determined: AST (E.C. 2.6.1.1) by means of Biochemtest kit (POCh Gliwice, Poland; cat. no. nr kat. 178 116 142), ALT (E.C.2.6.1.2.) by means of Biochemtest kit (POCh Gliwice, Poland; cat. no. 178 114 141), glutamate GLDH (E.C. 1.4.1.2.) by applying the spectrophotometric method *(6)*. Also determined were the activity of selected enzymes of the urea cycle: OCT-ase (E.C. 2.1.3.3) *(7)* and arginase (E.C. 3.5.3.1) *(8)*. The activity of enzymes in the liver was calculated per protein, which, in turn, was determined using the method of Lowry et al. *(9)*. Statistical analysis was performed with Statistic 6.0 PL software. Statistical methods included mean and standard deviation (SD). Mann–Whitney *U* test was used for comparison of data. A value of $p < 0.05$ was considered to be significant. The liver was taken for pathomorphological examinations, fixing it in 10% formalin. Pathomorphological changes in the liver were assessed on the basis of preparations made using the ordinary paraffin method, stained with hematoxylin and eosin. Pathomorphological changes and lesions were assessed using light microscope (magnification ×100, ×200, ×400, ×600). Microphotographs were taken by means of a Docuvel microscope, with an appliance for taking photographs by Carl-Zeiss-Jena.

RESULTS

The results are shown in Tables 1 (blood serum) and 2 (liver). As indicated in Table 1, the concentration of fluoride ions in both study groups

Table 2
AST, ALT, OTC-ase, Arginase, and GLDH Activity in Rat Liver

Groups	Activity														
	AST [IU/mg protein]			ALT [IU/mg protein]			OTC-ase [μmol cytruliny/ min/g protein]			Arginase [μmol mocznika/ min/g protein]			GLDH [IU/g protein]		
	X	SD	p	X	SD	p	X	SD	p	X	SD	p	X	SD	p
group I	2.18	0.35	-	0.92	0.21	-	97.74	17.57	-	3.40	0.49	-	119.3	26.00	-
group II	2.12	0.22	0.262	0.99	0.14	0.631	89.35	44.47	0.631	4.19	0.73	0.055	115.0	23.53	0.631
group III	1.97	0.42	0.337	0.89	0.18	0.337	87.11	40.55	0.150	4.48	2.47	0.262	123.3	26.30	0.873

increased 1.5 times in comparison with the control group. The results for the study groups are statistically significant. The concentration of glucose in the group administered F^- increased by 12%, without statistical significance, whereas in the group given F^- + caffeine increased by 17% (result without statistical significance).

The concentration of urea did not change in both study groups in comparison with the control group. The activity of AST (Table 2) in the liver decreased in the group given F^- + caffeine by 10% (result without statistical significance) in comparison with the control, whereas the activity of ALT in both study groups revealed no changes in relation to the control group.

The results concerning the determination of GLDH in the liver show no difference in the two study groups in comparison with the control group, whereas the activity of arginase in group II increased by 23% in comparison with the control group and increased by 31% in the group III (results without statistical significance).

Microphotograps of the Liver

In the assessment of lesions of the liver exposed to F^- and F^- + caffeine, the following criteria were applied:

1. Level of development and morphological character of retrogressive changes in hepatocytes
2. Assessment of hepatic connective tissue

In the microphotographs of liver specimens, no pathomorphological changes were observed in hepatocytes of both study groups. In group I, on liver examination in three animals, slight inflammatory infiltrations were observed within the triad, region, whereas in group III the only change noted was the presence of infiltrations in the hepatic triad region, with severity similar to that in the case of group I.

DISCUSSION

The increase of fluoride concentrations in blood serum caused by the administration of low and high doses of fluorine is a recognized phenomen

reported by many authors *(10,11)*. This is connected with the fact that fluoride ions are 100% absorbed by the intestine. In the reported experiment, we also noted the increase of fluoride ion concentrations in both experimental groups, yet caffeine did not contribute to the increased concentration of F^- in blood serum. On the other hand, the concentration of glucose in blood serum varies and is a complex phenomen on depending on the following:

1. Supply of carbohydrates in foodstuffs
2. Intensification of glucogenetic processes (formation of glucose of glucogenic substrates, mainly in the liver and kidney) as well as glycogenolytic or glycogenetic ones
3. Degree of glucose consumption by specific body organs
4. Possible loss of glucose with urine

In our experiment, we found a statistically insignificant increase of glucose concentration in blood serum, by 12% (group with F^-) and by 17% (group with F^- + caffeine). The increase of glucose concentration influenced by F^- is known. It is connected with the concentration of NaF administered, although even with low doses of F^- the concentration of glucose in serum might increase *(12)*. It is probable that fluorides intensify the glucogenetic processes *(13)*.

Urea, in turn, is the main final metabolite in the metabolism of proteins, and its concentration in blood serum depends on the decomposition of endogenous proteins or the excretory activity of kidneys. Only with the glomerular filtration (rate) decreased by over 50% does the concentration of urea in blood serum increase, which we noted in the case of high doses of NaF *(14)*.

During this experiment, for the relatively low doses of F^- administered to rats and in case of the group receiving F^- + caffeine, no changes has been observed concerning urea concentration. This might testify to the absence of kidney lesions for the applied concentrations of F^-.

Furthermore, the determination of activity of protein metabolism enzymes in liver requires that the hepatocytes function correctly (results of histopathological examinations), whereas the urea cycle in liver might be disturbed (increased activity of arginase). This can be connected with the increased turnover of the urea cycle and perhaps metabolic activation influenced by caffeine. The confirmation of slightly changed functioning of the liver might come from the noted infiltration in the hepatic triad area in F^- and F^- + caffeine study group. This phenomenon migth be attributed to the increased amount (acumulation) of F^- in the liver, as that organ, like the kidneys, is most prone to NaF *(15–17)* and binds that compound.

CONCLUSION

In this study, we found the following:

1. In blood serum, the applied doses of F^- (4.9 mg/kg body mass/d) and F^- + caffeine (4.9 mg F^-/kg body mass/d + 3 mg

caffeine/kg body mass/d) resulted in a statistically significant increase of the fluoride ion concentration in rat blood serum, a slight increase of glucose concentration (without statistical significance), and no changes in the concentration of urea.
2. In the liver: no change was observed in the functioning of hepatocytes; however, slight disturbances have been noted in the functioning of that organ, connected with the activation of urea cycle and cumulation of F^- (infiltration in hepatic triad area).

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