

DISTURBANCE OF PROTEIN METABOLISM IN RATS AFTER ACUTE POISONING WITH SODIUM FLUORIDE

E Birkner,^a E Grucka-Mamczar,^a Z Machoy,^b R Tarnawski,^a R Polaniak^a
Katowice and Szczecin, Poland

SUMMARY: Six-week-old male Wistar FL strain rats were given a single intraperitoneal injection of 35 mg of sodium fluoride/kg of body mass. After 90 min, the rats were killed to determine the concentration of fluoride, ammonia and urea in their blood serum along with the activity of glutamate dehydrogenase (GLDH) in their liver homogenates. A statistically significant increase in the concentration of urea and fluoride in the blood serum was found, and the activity of GLDH was elevated significantly in the liver. The results indicate increased deamination of amino acids in the liver and lesions in the kidney.

Keywords: Acute fluoride intoxication, Deamination, Glutamate dehydrogenase, Kidney lesions, Liver disturbances, Protein metabolism, Rat liver, Serum fluoride, Serum urea, Sodium fluoride.

INTRODUCTION

Most acute poisonings in humans caused by fluorides, as described in the literature, are associated with suicidal or accidental deglutition of fluorine-containing preparations, for example, insecticides. Most often, poisoning resulted from the intake of sodium fluoride, sodium fluosilicate, fluosilicic acid or hydrogen fluoride. Acute poisoning can terminate in death due to blocking cell metabolism since fluorides inhibit enzymatic processes, particularly metalloenzymes responsible for important vital processes.¹

The inhibiting action of fluoride affects Mg-dependent enzyme activity connected with energy formation both through oxygenic and nonoxygenic pathways. Fluorides thus decrease the pool of intracellular ATP (adenosine triphosphate).^{2,3} In the oxygenic pathway, fluorides inhibit electron-transporting systems (especially cytochromes) and some Krebs cycle enzymes.^{4,5}

In glycolysis, the fluoride ion impedes the key enzyme of this path, *i.e.* pyruvate kinase, which participates directly in ATP formation.⁶ Combustion of fatty acids is also inhibited since the fluoride ion blocks Mg-dependent pyrophosphatase.⁷ An increase in the biosynthesis of lipids during experimental fluorosis can occur as well.^{8,9}

Fluoride ion affects not only the fat and carbohydrate balance but also protein equilibrium. Furthermore, as a result of the action of fluoride ion on the human organism, changes in proteometabolism become pronounced last of all.

In this study we investigated how much protein metabolism is altered in rats after a large single dose of sodium fluoride. Our plan was to study the concentration of fluoride ions, ammonia and urea in the blood serum and the activity

^aFor Correspondence: Dr Ewa Birkner, Department of Biochemistry, Silesian Academy of Medicine, 41-808 Zabrze, Jordan Str. 19, Katowice, Poland.
E-mail: biochemz@infomed.slam.katowice.pl; ^bDepartment of Biochemistry and Chemistry, Pomeranian Academy of Medicine, Szczecin, Poland.

of glutamate dehydrogenase (GLDH) (EC1.4.1.2.) in the liver, since these biochemical parameters are indirectly related to proteometabolism.

MATERIALS AND METHODS

The experiments were conducted on 18 6-week-old male Wistar FL strain rats obtained from the Central Test Animal Quarters at the Silesian Academy of Medicine in Katowice. The animals were divided into two groups: a control group of 10 rats and a test group of 8. The mean body mass of these animals was $202.8 \text{ g} \pm 15.02 \text{ g}$.

The rats in the test group were treated intraperitoneally with a single injection of sodium fluoride (35 mg/kg of body mass) in 0.5 mL of physiological saline (0.9% NaCl). Animals in the control group received intraperitoneally 0.5 mL of physiological saline.

After 90 min the rats were anesthetized with ether to collect blood from the left ventricle of the heart. The concentration of fluoride in the blood serum was determined using a fluoride ion selective electrode, the urea concentration by means of an enzymatic kit from ALPHA-DIAGNOSTICS Firm (Germany), Cat. No. B 6550, and the ammonia concentration by the kit from SIGMA-DIAGNOSTICS Company (USA), Cat. No. 171-A.

Livers were rinsed with a chilled 0.9% NaCl solution and, after isolation, a 10% (w/v) homogenate was prepared in physiological saline. Upon centrifuging ($1750 \times g$ for 10 min), the activity of glutamate dehydrogenase (GLDH) was determined by the spectrophotometric method of Krawczyński.¹⁰ The enzyme activity was calculated per gram of protein according to the directions of Lowry *et al.*¹¹

RESULTS

After the single intraperitoneal NaF injection, a statistically significant increase in the concentration of fluoride and urea was observed in the blood serum of the test rats under examination. The concentration of fluoride in the blood serum in these rats was about 26 times higher than that of the control group. The concentration of urea in the rat blood serum also rose significantly ($p < 0.001$) and symptomatically by 80.36%. The ammonia concentration assayed in the blood serum showed a small nonsignificant ($p > 0.2$) upward trend of 7.89% (Table 1).

TABLE 1. Concentration of fluoride, urea and ammonia in rat blood serum after a single intraperitoneal injection of 35 mg NaF/kg of body mass

Parameter mmol/L	Control group		Test Group		p
	X	SD	X	SD	
Fluoride	0.0044	0.0002	0.117	0.015	$p < 0.001$
Urea	25.987	3.385	46.871	3.509	$p < 0.001$
Ammonia	0.285	0.04696	0.3075	0.031545	$p > 0.2$

*Nonsignificant

The GLDH activity in the hepatic homogenates of the test group increased statistically ($p < 0.05$) and symptomatically by 48% compared with that of the control group (Table 2).

TABLE 2. Activity of GLDH in rat liver after a single intraperitoneal injection of 35 mg NaF/kg of body mass

Group of Rats	GLDH Activity U/g of protein		
	X	SD	p
Control group	4.012	1.89	-
Test group	5.941	1.68	$p < 0.05$

DISCUSSION

As is well known, amino acids that occur in excess in an organism during protein synthesis cannot be stored, and they are therefore degraded. Their catabolism includes transamination and oxidative deamination catalysed by glutamate dehydrogenase (GLDH) that withdraws free ammonia.

Together with asparaginate, the resulting free ammonia is a nitrogen source for the synthesis of urea within the hepatic urea cycle. Since GLDH activity is controlled allosterically, GTP and NADH provide the allosteric inhibitors, whereas GDP and ADP are the activators.

A reduction of highly energetic compounds thus accelerates the oxidation of amino acids. The growth of GLDH activity within hepatic tissue observed in our study might therefore be a consequence of reducing the quantity of high-energy compounds. Such a phenomenon often takes place under the influence of fluorides, the more so since fluorides interfere with proteins of the respiratory chain,¹² and can increase deamination of amino acids in the liver by the action of an acute dose as administered here.

The synchronous increase in GLDH activity in the liver and the increase in urea concentration in the blood serum by fluoride in the test rats can also be seen to support the activation of urea cycle enzymes. However, from our earlier studies,¹³ it follows that enzyme activities of the urea cycle in rats are subject to a significant inhibition but not an increase under the influence of NaF (10 and 30 ppm F⁻) in the drinking water.

As in our findings here, Guzman *et al*¹⁴ also found an increase in the urea content in blood plasma of rats and mice treated intraperitoneally with Irloxacin, a fluorine-containing antibiotic.

Because the ammonia concentration in the blood serum of the test rats showed only a small increase, elevation of urea in serum could result rather from defective excretory function of the kidneys, especially since increased

urea concentration in blood serum is, as a general rule, caused by renal insufficiency, with or without obstruction of urinary tracts. Similar findings are reported by Appleton,¹⁵ who administered 0.4 mmol of sodium fluoride/kg of body mass to rats as an intraperitoneal injection and found an increase in urea concentration. He explained the high serum urea concentration by lesions of the kidneys. This view is shared by others, including Monsour and Kruger,¹⁶ who also report kidney lesions from fluorides. Such kidney damage in our rats could result from the large concentrations of fluoride in the blood serum (about 26 times larger than in the control group).

In summary the changes observed by us provide evidence for disruption of protein metabolism in rats exposed to an acute dose of NaF. Tyrtysnikov and Pedik¹⁷ also found disturbances in protein metabolism in rat liver during experimental anemia induced by NaF. Likewise, it can be assumed that the NaF dose used here caused kidney lesions in our test rats, since high concentrations of fluoride in plasma amounting to over 90 $\mu\text{mol/L}$ lead to kidney lesions,¹⁸ or incur significant animal dehydration.

REFERENCES

- 1 Indulski JA, editor. Kryteria zdrowotne środowiska. Fluor i fluorki tom 36, (Sanitary criteria of environment. Fluorine and fluorides) Vol. 36. Warszawa: Państwowy Zakład Wydawnictw Lekarskich 1989;87.
- 2 Chitra T, Ramana Dao JV. Preliminary study of the unit oxygen consumption of *Charma punctatus* on exposure to NaF. *Fluoride* 1984;17:105-7.
- 3 Singh M. Biochemical and cytochemical alterations in liver and kidney following experimental fluorosis. *Fluoride* 1984;17:81-93.
- 4 Machoy Z. Effect of fluorine compounds on the respiratory enzymes. *Fluoride* 1985;18:234 (abstract).
- 5 Miller GW, Eged MN, Shupe JL. Aconitate hydratase activity and citrate content of heart and kidney in fluoride affected cows. *Fluoride* 1978;11:14-7.
- 1 Gumińska M, Sterkowicz J. Effect of sodium fluoride on glycolysis in human erythrocytes and Erlich ascites tumor cells in vitro. *Acta Biochim Pol* 1976; 23:285-91.
- 7 Batenburg J, Bergh SG. The mechanism of inhibition by fluoride of mitochondrial fatty acid oxidation. *Biochim Biophys Acta* 1972;280:495-505.
- 8 Shashi A. Biochemical effects of fluoride on lipid metabolism in the reproductive organs of male rabbits. *Fluoride* 1992;25:149-54.
- 9 Machoy-Mokrzyńska A, Put A, Ceglecka M, Myśliwiec Z. Influence of essential phospholipids (EPL) on selected biochemical parameters of lipid metabolism in rats chronically exposed to ammonium fluoride vapours. *Fluoride* 1994;27:201-4.
- 10 Krawczyński J. Editor. Diagnostyka enzymologiczna w medycynie praktycznej (Enzymologic diagnostics in practical medicine), Warszawa: Państwowy Zakład Wydawnictw Lekarskich 1972;85-7.
- 11 Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *J Biol Chem* 1951;193:265-75.

- 12 Ogoński T, Wieczorek P, Paszkiewicz E, Machoy Z. Transport jonów fluorkowych przez wewnętrzną błonę mitochondrialną (Fluoride ion transport through intramitochondrial membrane) In: Machoy Z, Samujło D, editors. *Metabolizm fluoru '92 (Fluorine Metabolism '92)*, V Fluorine Symposium, 1992; Sep. 25, 92-3.
- 13 Grucka-Mamczar E, Machoy Z, Tarnawski R, Birkner E, Mamczar A. Influence of long-term sodium fluoride administration on selected parameters of rat blood serum and liver function. *Fluoride* 1997;30:157-64.
- 14 Guzman A, Garcia C, Demestre I. Acute and subchronic toxicity studies of the new quinolone antibacterial agent irloxacin in rodents. *Arzneimittelforschung* 1999;49:448-56.
- 15 Appleton J. Changes in the plasma electrolytes and metabolites of the rat following acute exposure to sodium fluoride and strontium chloride. *Arch Oral Biol* 1995;40:265-8.
- 16 Monsour PA, Kruger BJ. Effect of fluoride on soft tissues in vertebrates (a review). *Fluoride* 1985;18:53-61.
- 17 Tyrtysnikov IM, Pedlik VP. Sodium fluoride influence upon energy and protein liver metabolism after its experimental ischemia. *Fiziol Zh (Moscow)* 1992;38:42-6 (abstract in *Fluoride* 1992;25:196).
- 18 Gumińska M. Związki fluoru w środowisku i ich wpływ na zdrowie. (Fluorine compounds in the environment and their effect on health). In: Gumińska M, editor. *Chemiczne substancje toksyczne w środowisku i ich wpływ na zdrowie człowieka. (Chemical toxic substances in environment and their effect on human health)*. Wrocław, Warszawa, Kraków: Zakład Narodowy im. Ossolińskich, Wydawnictwo Polskiej Akademii Nauk 1990;59-81.