FLUORIDE EFFECTS ON GLUTATHIONE PEROXIDASE AND LIPID PEROXIDATION IN RATS

I Inkielewicz,[a] J Krechniak[a,b]
Gdańsk, Poland

SUMMARY: Eight-week old male Wistar rats weighing 180 g were given sodium fluoride in drinking water at a concentration of 5 and 25 mg F^-/L for 12 weeks. Control animals received tap water containing 0.3 mg F^-/L. The activity of glutathione peroxidase and the concentration of malondialdehye (MDA) were determined in kidney, liver, brain, testis, and blood or plasma. In exposed animals the activity of glutathione peroxidase decreased significantly, and the concentration of MDA increased in a dose and exposure-time dependent manner. The results of this study confirm other reports that fluoride induces free radical toxicity in animals.

Keywords: Antioxidant potential; Fluoride and rats; Fluoride exposure; Glutathione peroxidase; Lipid peroxidation; Malondialdehyde.

INTRODUCTION

Repeated exposure to fluoride (F^-) from fluorine compounds may involve metabolic pathways associated with lipid, carbohydrate, bone, and energy metabolism. Fluoride also inhibits the activity of many enzymes. Its adverse action on the structure and function of many organs suggests that fluoride may generate free radicals and in consequence interfere with antioxidant defence mechanisms in the living cell.[1,2]

As reviewed recently in *Fluoride*,[2,3] the impact of fluoride on free radical parameters has been investigated by various authors. A number of studies indicate that fluoride induces the generation of ROS (reactive oxygen species) and affects lipid peroxidation accompanied by a decline in activities of some antioxidant enzymes. A decreased GSH/GSSG (reduced glutathione/oxidized glutathione) ratio and an increased TBARS (thiobarbituric acid reactive substances) production were reported in rats exposed to 12 mg F^-/L in drinking water during 15 days.[4] Guan *et al*[5] reported a decrease in glutathione peroxidase (GPx) activity and GSH level in erythrocytes and an increase in lipid peroxidation in serum of rats given 10 or 30 mg F^-/L in drinking water for 8 months. Shivarajashankara *et al*[1] found higher levels of GSH and malondialdehyde (MDA) as well as lower activity of superoxide dismutase in plasma of rats receiving 100 mg F^-/L in drinking water during 4 months. The concentration of GSH and MDA and the activity of glutathione S-transferase were also elevated in brain and liver.

[a]Department of Toxicology, Medical University of Gdańsk. [b]For Correspondence: Dept. of Toxicology, Medical University of Gdańsk. 80–416, Gdańsk, Al. Gen. Hallera 107, Poland, E-mail: woczar@amg.gda.pl

Results obtained by different authors are sometimes contradictory. Most of the investigations were performed using only one exposure level (usually relatively high, *i.e.*, 50–150 mg F^-/L) and were confined to one or two tissues. Therefore the aim of this study was to investigate the impact on blood, liver, kidney, brain, and testis of subchronic exposure to sodium fluoride administered to rats in drinking water at two different concentrations.

MATERIALS AND METHODS

The investigation was performed on male Wistar rats 8 weeks old weighing 180 g given sodium fluoride in drinking water at a concentration of 5 and 25 mg F^-/L for 12 weeks. Control animals received tap water containing 0.3 mg F^-/L. At the beginning of the experiment and after 2, 4, and 12 weeks of exposure, 6 animals from each group were sacrificed, and samples of blood, liver, kidney, brain, and testis were collected. In this material glutathione peroxidase (GPx) activity was determined by the method of Paglia and Valentine.[6] The accuracy of measurement was tested with the reference material RANSEL SC 692 (Randox). Malondialdehyde (MDA) concentration was determined by the method of Rice-Evans *et al*.[7] Protein content was determined by the method of Lowry *et al*.[8]

Statistical analysis was performed using Fisher-Snedecor and Student's t tests.

RESULTS AND DISCUSSION

Effects on glutathione peroxidase (GPx) activity are presented in Table 1. Malondialdehyde (MDA) levels are presented in Table 2.

Antioxidant protection of living organisms consists of several levels of defensive response activity including enzymes, proteins, and low-molecular-mass agents. Among enzymatic mechanisms of antioxidant protection, GPx has an important role that is specific for GSH as a hydrogen donor. It is a homotetramer consisting of four 22 kDa subunits, each with one selenocysteine residue. The enzyme is found essentially in cytosol and mitochondria of all tissues and reduces hydrogen peroxide and some organic hydroperoxides to water and alcohols, respectively.

In our experiment we observed a decrease in GPx activity in all investigated tissues: blood, liver, kidney, brain, and testis. The decrease was both dose and exposure time-dependent. In blood, kidney, and brain the decrease was significant after 4 weeks of exposure in rats receiving 25 mg F^-/L and after 12 weeks in the lower exposure group. In liver and testis the decrease was already significant already after 2 weeks of exposure in rats given the higher dose, and after 4 weeks in both groups given NaF.

Table 1. GPx activity in soft tissues (U/g protein) and blood (U/g Hb) of rats exposed to Na F in drinking water (values are means ± SD; numbers of animals in parentheses)

Tissue		Exposure time (weeks)			
		0	2	4	12
Kidney	Controls	653 ±40.5 (5)	644 ± 126 (6)	667 ± 59.7 (6)	668 ± 32.4 (6)
	5 mg F^-/L		652 ± 11.1 (5)	626 ± 61.7 (5)	572 ± 28.8‡ (5)
	25 mg F^-/L.		597 ± 14.2 (6)	553 ± 14.1† (5)	528 ± 38.9‡ (6)
Liver	Controls	1930 ± 45.4 (5)	1920 ± 7.9 (6)	1930 ± 66.6 (6)	2020 ± 67.9 (5)
	5 mg F^-/L		1910 ± 275 (4)	1640 ± 75.0‡ (5)	1590 ± 43.1‡ (5)
	25 mg F^-/L.		1630 ± 35.9‡ (6)	1430 ± 121‡ (6)	1450 ± 114‡ (6)
Brain	Controls	508 ± 7.0 (5)	508 ± 74.3 (6)	426 ± 135 (5)	433 ± 50.9 (6)
	5 mg F^-/L		457 ± 111 (6)	358 ± 68.4 (5)	353 ± 60.2* (6)
	25 mg F^-/L.		410 ± 67.8 (6)	285 ±14.2* (5)	264 ± 39.9‡ (6)
Testis	Controls	268 ± 9.2 (4)	271 ± 91.7 (6)	295 ± 6.4 (6)	283 ± 45.6 (6)
	5 mg F^-/L		253 ± 42.1 (6)	224 ± 39.0† (6)	192 ± 25.4† (6)
	25 mg F^-/L.		196 ± 24.0* (6)	182 ± 11.7‡ (6)	175 ± 25.1‡ (6)
Blood	Controls	60.8 ± 8.9 (5)	57.4 ± 8.3 (4)	55.7 ± 6.4 (6)	57.0 ± 1.7 (5)
	5 mg F^-/L		58.7 ± 5.8 (6)	50.4 ± 3.2 (6)	50.3 ± 6.0* (5)
	25 mg F^-/L.		55.0 ± 5.3 (6)	44.0 ± 5.7† (6)	46.5 ± 1.1‡ (5)

*$p < 0.05$. †$p < 0.01$. ‡$p < 0.001$. Where nothing is indicated, differences from the controls are nonsignificant.

We also found that the activity of GPx correlated well ($p<0.001$) with the level of GSH in all the tissues investigated.[9] The correlation coefficients were: blood ($r = 0.844$), brain ($r = 0.837$), kidney ($r = 0.826$), liver ($r = 0.745$), and testis ($r = 0.697$).

These results are consistent with data reported by Guan *et al*[5] in erythrocytes of rats given 10 or 30 mg F^-/L in drinking water for 8 months, by Liu *et al*[10] in liver of rats given 150 mg NaF/L for 6 months, and by Guo *et al* [11] in liver of rats given 50, 100, and 150 mg NaF/L for 3 months.

Table 2. MDA content in soft tissues and serum (NM/g protein) of rats exposed to NaF in drinking water (values are means ± SD for 6 animals)

Tissue		Exposure time (weeks)			
		0	2	4	12
Kidney	Controls	880 ± 13.9	888 ± 8.8	877 ± 65.0	1150 ± 122
	5 mg F^-/L		1061 ± 149*	1160 ± 89.4‡	1680 ± 151‡
	25 mg F^-/L.		1195 ± 155‡	1450 ± 94.2‡	1900 ± 80.6‡
Liver	Controls	501 ± 6.5	496 ± 13.3	526 ± 41.9	551 ± 42.1
	5 mg F^-/L		529 ± 46.2	589 ± 58.4	731 ± 23.0‡
	25 mg F^-/L.		611 ± 16.7‡	713 ± 51.9‡	955 ± 83.8‡
Brain	Controls	963 ± 10.9	967 ± 66.7	1110 ± 111	1390 ± 87.4
	5 mg F^-/L		1022 ± 95.0	1250 ± 141	2190 ± 381‡
	25 mg F^-/L.		1250 ± 114‡	1560 ± 111‡	2680 ± 167‡
Testis	Controls	500 ± 14.0	511 ± 44.0	539 ± 89.4	660 ± 66.8
	5 mg F^-/L		577 ± 41.7	660 ± 59.0*	832 ± 63.2†
	25 mg F^-/L.		705 ± 98.7†	869 ± 81.8‡	1060± 92.4‡
Plasma	Controls	344 ± 7.3	341 ± 25.8	355 ± 36.4	386 ± 20.9
	5 mg F^-/L		440 ± 23.7‡	570 ± 26.4‡	554 ± 17.3‡
	25 mg F^-/L.		486 ± 27.1‡	602 ± 35.5‡	606 ± 12.8‡

*$p<0.05$. †$p<0.01$. ‡$p < 0.001$. Where nothing is indicated, differences from the controls are nonsignificant.

The most common free radical-mediated tissue impairments are: lipid peroxidation, oxidation of proteins, and oxidative DNA damage. Lipid peroxidation is usually evaluated by the thiobarbituric acid test. Many authors[1,4,5,10–12] report higher MDA content in animals exposed to fluoride in different experiments. In our study an increase in MDA concentration in all investigated tissues was observed. The increase was both dose and exposure time-dependent. In plasma, kidney, and testis the increase was already statistically significant in both intoxicated groups of animals after 2 weeks of exposure and lasted until the end of the experiment. In liver and brain the MDA

increase in rats receiving the lower dose was significant only after 12 weeks of exposure, but in animals given the higher dose it was significant after 2 weeks of exposure.

We also noticed an impact of fluoride on oxidation of proteins using the carbonyl test. A dose and exposure time-dependent increase in the concentration of carbonyl groups was found in all investigated tissues of rats exposed to 5 and 25 mg F^-/L in drinking water.[9]

The results of this study are consistent in all the tissues we examined at both F^- exposure levels, and they confirm those of many authors that fluoride can exert its toxic action through free radical-mediated mechanisms. However, we note that some authors[13,14] report that fluoride does not impair antioxidant systems. Discrepancies in the results obtained by others are possibly due to many factors like differences in animal species, dose, mode and time of exposure, and kind of tissue examined as well as methods used for biochemical assay.[2,3] Nevertheless, in our view most reports favour the oxidative stress theory of fluoride.

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