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FLUORIDE CONTENT IN SOFT TISSUES AND URINE OF RATS EXPOSED TO SODIUM FLUORIDE IN DRINKING WATER

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SUMMARY: Eight-week old male Wistar rats weighing about 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 fluoride content in liver, kidney, brain, testis, and serum was determined at the beginning of the experiment and after 2, 4, and 12 weeks of exposure. Urinary fluoride concentration was determined weekly. In all the tissues and organs the fluoride content increased in a dose-dependent and a time-dependent manner. In animals receiving the higher dose of sodium fluoride the increase after 12 weeks of exposure was about two-fold in serum, seven-fold in liver and kidney, nine-fold in brain, and twelve-fold in testis. Urinary fluoride also increased from the beginning of exposure in a dose-dependent manner.

Keywords: Fluoride exposure; Fluoride in brain; Fluoride in kidney; Fluoride in liver; Fluoride in serum; Fluoride in testis; Rats; Urinary fluoride.

INTRODUCTION

The concentrations of fluoride in soft tissues are not well known, and only limited data on this subject are available.[1-5] Moreover, some of the data come from times when less accurate analytical methods were used. Over 30 years ago, one of us determined fluoride concentrations in tissues and body fluids of rats and rabbits.[1] Under most conditions fluoride concentrations in soft tissues are low, usually at the part per million level.

Fluoride when absorbed is rapidly distributed by systemic circulation into the intracellular and extra- cellular water of tissues. More than 90% of the total body burden is retained in bones and teeth. The concentration of fluoride in soft tissues is reflected by that in blood. Fluoride is distributed from the plasma to all tissues and organs. The rates of delivery are generally determined by the blood flow to the tissues in question. Consequently, steady-state fluoride concentrations are achieved more rapidly between plasma and well-perfused tissues, such as liver and kidney. The major route for the removal of fluoride from the body is by the kidney. Urinary fluoride is regarded as the best indicator of exposure to fluorine compounds, and usually it correlates well with the level of fluoride in drinking water.[6]

Fluoride in soft tissues is associated with structural changes and disorders in their function.[7-10] The determination of fluoride and its distribution in soft tissues of humans and animals may therefore be of practical significance.

The aim of this study was to determine the fluoride level in liver, kidney, brain, testis, serum, and urine of rats exposed to NaF in drinking water.

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MATERIALS AND METHODS

Eight-week old male Wistar rats weighing about 180 g were given sodium fluoride in their 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. All animals were fed a standard laboratory pellet diet containing 0.7 mg water-extractable F/kg. At the beginning of the experiment and after 2, 4, and 12 weeks of exposure, six animals of each group were sacrificed, and samples of blood, liver, kidney, brain, and testis were collected. The concentration of fluoride in soft tissues was determined after dry combustion of the sample according to the method described recently by us.[11] Every week the concentration of fluoride in urine was determined directly after dilution with equal volumes of TISAB buffer by a fluoride-specific electrode (Orion) against a Ag/AgCl reference electrode.[6]

The accuracy of measurements was assessed with reference materials – in serum and soft tissues with Serum Control (Clin Check), and in urine with Seronorm Control Urine (Nycomed Pharma AC, Oslo). Mean fluoride recovery was 99.4% from urine, 98.8% from serum, and 104% from soft tissues. The levels obtained were within the assayed 5% confidence levels. Statistical analysis was performed using the Fisher-Snedecor and Student's t-test.

RESULTS AND DISCUSSION

Results of the fluoride determinations in liver, kidney, brain, testis, and serum are presented in Table 1. Urinary fluoride concentrations corrected to normal specific gravity are presented in Table 2.

In soft tissues of control animals the highest mean fluoride concentration occurred in kidney tissue, which is consistent with data reported by other authors.[3,4] The kidney represents the major route for the removal of fluoride from the body, and fluoride is concentrated to much higher levels in the kidney tubules than it is present in plasma. Consecutively lower fluoride concentrations were found in liver, brain, testis, and serum.

After exposure to fluoride, the fluoride content in all the investigated organs and tissues increased in a dose-dependent and exposure-time-dependent manner. The highest fluoride concentrations were always found in kidney and the lowest in serum. In the remaining tissues – liver, brain, and testis – fluoride concentrations were similar at an intermediate level. In comparison with the control animals, the relative increase of fluoride in soft tissues during subchronic exposure (12 weeks) in the animals receiving the higher dose of sodium fluoride was the highest in testis and the lowest in liver. The significant increase of fluoride in soft tissues as a result of higher exposure to fluoride may explain the negative impact of fluoride on cellular structures and activity of many enzymes in the investigated tissues.

Table 1. Fluoride content in soft tissues (μg F^-/g) and serum (μg F^-/mL) of rats exposed to NaF in drinking water

Tissue	Exposure level	Exposure time (weeks) 0	2	4	12
Kidney	controls	0.943 ± 0.101	0.955 ± 0.062 (6)	1.43 ± 0,061 (6)	1.65 ± 0.171 (5)
	5 mg F^-/L	(6)	2.72 ± 0.102 (6)	4.72 ± 0,644 (6)	6.20 ± 0.184 (5)
	25 mg F^-/L		4.20 ± 0.099 (6)	6.04 ± 0,473 6)	7.11 ± 0.230 (5)
Liver	controls	0.771 ± 0.079	0.833 ± 0.074 (6)	0.918 ± 0.108 (6)	1.35 ± 0.121 (6)
	5 mg F^-/L	(6)	2.19 ± 0.165 (6)	3.26 ± 0.368 (6)	4.03 ± 0.537 (6)
	25 mg F^-/L		3.25 ± 0.092 (6)	4.44 ± 0.187 (6)	5.17 ± 0.325 (6)
Brain	controls	0.610 ± 0.020	0.653 ± 0.092 (6)	0.826 ± 0.058 (6)	1.22 ± 0.052 (5)
	5 mg F^-/L	(6)	2.15 ± 0.122 (6)	3.14 ± 0.233 (6)	4.63 ± 0.077 (5)
	25 mg F^-/L		2.91 ± 0.157 (6)	4.23 ± 0.127 (6)	5.17 ± 0.043 (5)
Testis	controls	0.505 ± 0.028	0.896 ± 0.045 (6)	0.867 ± 0.044 (6)	1.20 ± 0.108 (6)
	5 mg F^-/L	(6)	2.40 ± 0.149 (6)	3.30 ± 0.205 (6)	5.01 ± 0.108 (6)
	25 mg F^-/L		3.54 ± 0.195 (6)	4.26 ± 0.188 (6)	5.92 ± 0.078 (6)
Serum	controls	0.051 ± 0.002	0.059 ± 0.005 (4)	0.040 ± 0.002 (5)	0.042 ± 0.007(5)
	5 mg F^-/L	(5)	0.056 ± 0.008*(4)	0.058 ± 0.003 (5)	0.091 ± 0.010(5)
	25 mg F^-/L		0.107 ± 0.009 (4)	0.080 ± 0.016 (5)	0.095 ± 0.010(5)

Control animals received drinking water containing 0.3 mg F^-/L. Values are means ± SD. Numbers of animals are given in parentheses. All results from tissues and serum of exposed animals except one (*) differ significantly from controls ($p<0.001$).

Table 2. Fluoride content (mg F^-/L) in urine adjusted to normal specific density of rats exposed to sodium fluoride in drinking water

Exposure time in weeks	Fluoride content adjusted to normal urine density (1.024 g/mL) Animals		
	Controls 0.3 mg F^-/L	Exposed to 5 mg F^-/L	Exposed to 25 mg F^-/L
0	1.33 ± 0.042	1.33 ± 0.042	1.33 ± 0.042
1	1.62 ± 0.219	2.97 ± 0.034	4.05 ± 0.961
2	1.61 ± 0.713	3.52 ± 0.250	4.63 ± 0.341
3	1.49 ± 0.112	4.49 ± 0.294	5.34 ± 0.052
4	1.69 ± 0.698	5.57 ± 0.611	6.03 ± 0.565
5	2.22 ± 0.352	4.99 ± 0.672	6.96 ± 0.170
6	1.95 ± 0.094	4.51 ± 0.465	6.64 ± 1.827
7	1.95 ± 0.147	5.64 ± 0.183	7.27 ± 0.067
8	1.77 ± 0.063	6.39 ± 0.338	8.64 ± 0.593
9	2.18 ± 0.014	5.64 ± 0.670	8.93 ± 0.054
10	1.94 ± 0.162	5.22 ± 0.703	7.69 ± 0.214
11	1.59 ± 0.394	4.35 ± 0.263	7.14 ± 0.367
12	1.83 ± 0.009	4.98 ± 0.147	6.06 ± 0.718

Values are means for four animals ± SD.

The concentration of fluoride in urine increased from the beginning of the exposure period in a dose-dependent manner. The highest fluoride levels were noticed after about two months of exposure when probably a balance between absorption, storage in bones, and excretion was achieved. The results of urinary fluoride determination proved that exposure proceeded as intended according to the conditions of the experiment.

In summary, the results of this study provide reference levels of fluoride in soft tissues and body fluids of rats as well as in rats exposed subchronically to sodium fluoride in drinking water.

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