EFFECTS OF FLUORIDE INGESTION ON SERUM LEVELS OF THE TRACE MINERALS Co, Mo, Cr, Mn, AND Li IN ADULT MALE MICE

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SUMMARY: The aim of this study was to determine long-term effects of oral fluoride (F) ingestion on serum Co, Mo, Cr, Mn, and Li levels in mice. Fifty adult male Swiss mice were divided into five equal groups and given tap water containing 0, 10, 20, 30, and 40 mg F/L. After four months, serum samples were collected via cardiac puncture under ether anesthesia. Mineral analyses were determined by atomic absorption spectrometry with a graphite furnace system. Serum Co levels increased ($p<0.05$), whereas Mo, Cr, and Mn levels decreased ($p<0.05$), and Li levels remained unchanged. F concentration in the water was positively correlated with serum Co ($r = 0.488$, $p < 0.001$) and negatively with serum Mo, Cr, and Mn, but not with Li ($r = 0.057$, $p = 0.695$).

Keywords: Chromium; Cobalt; Lithium; Manganese; Mice and fluoride; Molybdenum; Serum minerals; Trace minerals.

INTRODUCTION

Depending on the mineral composition of drinking water, fluoride (F) in drinking water is able to pass rapidly through the intestinal mucosa and interfere with major metabolic pathways of animals.[1] The resulting F intoxication disturbs energy pathways, protein processes, lipid metabolism, and parenchymatous organs and functions.[2-7]

Fluctuations in serum levels of Mo, Co, Cr, Mn, and Li have adverse health effects on mammals.[8-9] Because of its small size and high charge density, the F anion has a strong affinity for polyvalent cations.[10,11] Interactions between minerals are well documented,[9,12-14] and F interacts or combines with Ca, Mg, K, Cu, and Zn ions.[15-22] There is, however, a lack of information on interactions between F and Co, Mo, Cr, Mn, and Li. The aim of this study was to determine the effect of various levels of oral F intoxication in mice on the serum concentrations of Co, Mo, Cr, Mn, and Li.

MATERIALS AND METHODS

Animals and procedures: This study involved 50 clinically healthy male Swiss albino mice, aged 4 months, weighing 28.8 ± 1.18 g, that were divided into five equal groups: four experimental groups receiving F in their drinking water at 10, 20, 30, and 40 mg F/L plus a control group receiving only low-F tap water. The animals were fed a commercial animal food and given water *ad libitum* before and during the experiment. The commercial food was purchased from Bayramoğlu Yem ve Un San. Tic. A.Ş. ISO 9001:2000, ISO 22000:2005 with the composition and ingredients shown in Table 1.

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Table 1. Ingredients of diet given to 50 adult male Swiss mice

Diet composition	Units	Diet composition	Units
Dry matter	88%	Vitamin A	5000 IU/kg
Crude protein	17%	Vitamin D3	600 IU/kg
Crude cellulose	12%	Vitamin E	25 mg/kg
Crude ash	10%	Metabolic energy	2600 kcal/kg
Acid insoluble ash	1%	Cobalt	0.008 mg/kg dry matter
Calcium	1.5%	Molybdenum	0.82 mg/kg dry matter
Phosphorus	0.75%	Chromium	0.064 mg/kg dry matter
NaCl	0.6%	Manganese	7.3 mg/kg dry matter
		Lithium	1.9 mg/kg dry matter

Raw materials for this composition: barley, corn, corn chaff, corn gluten, wheat, rye chaff, cottonseed meal, sunflower meal, dicalcium phosphate, vitamins, and minerals.

The content levels of F (prepared from NaF, Merck 106449) in the drinking water of the mice were confirmed by an Orion 4 star pH-ISE portable device equipped with a F ion selective electrode (Thermo electron corporation, USA). Coefficients of variations (CV) of the device for 0, 10, 20, and 40 mg F/L were 1.12%, 1.23%, 2.18%, and 1.15%, respectively.

Blood samples and laboratory analyses: Blood samples were collected by cardiac puncture under ether anesthesia. Serum was separated by centrifugation at 3500 rpm for 15 min, and the mineral concentrations were measured by an atomic absorption spectrometer equipped with a graphite furnace system (GFAAS) (Thermo Elemental S4, Thermo Electron Corporation, Cambridge, UK).

For the mineral determinations, the serum was diluted in a ratio of 1/4 for Co, Cr, and Li and 1/10 for Mo and Mn. A deuterium background correction were used for all measurements. Extended life cuvettes were used for Co, Mo, Cr, and Mn. A normal electrographite cuvette was used for the Li analyses. As a matrix modifier, 50 µg of magnesium nitrate was used for 20-µL sample injections for Co, Cr, and Mn, but not for Mo and Li. Standard solutions of Co, Mo, Cr, Mn, and Li were supplied by Fluka Chemie GmbH, Switzerland (Fluka 02776, 69876, 27014, 02654, and 02685, respectively).

Accuracy control of GFAAS was assured by using previously prepared known-level standard solution (Fluka) for each mineral measurement. These solutions were aspirated 6 times per 10 samples during analyses. Coefficients of variations (CV) of these parameters were calculated from these findings with the following results: Co: 7.41%, Mo: 6.13%, Cr: 5.98%, Mn: 7.14%, and Li: 8.81%. All lab-ware was plastic material, and all solutions were prepared with distilled and deionized water.

Statistical analysis: Comparison of the treatment groups with the control group was made by 2-sample t test. The Pearson correlation was used to test the relationships between serum mineral levels and F content of drinking water. Simple regression analyses were used for observing the effect of oral F applications on serum Co, Mo, Cr, and Mn levels. Statistical analyses were performed using Minitab statistical software version 13.0.[23] Data are presented as means ± SE Mean.

RESULTS

As seen in Table 2, following F supplementation in the mice, the serum level of Co increased but serum levels of Mo, Cr, and Mn decreased. The serum level of Li however, remained constant. Co levels increased significantly only in the 40 mg-F/L group. Mo levels decreased significantly after 30 mg F/L and continued in the 40 mg F/L group. A decrease in the Cr level occurred in all treatment groups, but was not significant in the 10 mg F/L F group. Similarly, a significant decrease in Mn was also observed in 20 mg F/L group ($p<0.05$) and continued in the 30 and 40 mg F/L groups ($p<0.01$).

Table 2. Serum Co, Mo, Cr, Mn, and Li levels according to groups (values are means ± SE)

Minerals	Control	F concentration in the drinking water of the administered groups			
	0 mg F/L	10 mg/L	20 mg/L	30 mg/L	40 mg/L
Co (ng/mL)	0.330 ± 0.05	0.329 ± 0.02	0.346 ±0.04	0.372 ±0.05	0.408 ± 0.08†
Mo (ng/mL)	1.519 ± 0.17	1.445 ± 0.36	1.275 ± 0.36	0.858 ± 0.22‡	0.528 ± 0.23‡
Cr (ng/mL)	0.391 ± 0.04	0.375 ± 0.03	0.354 ± 0.02†	0.358 ± 0.03†	0.349 ± 0.04†
Mn (ng/mL))	0.961 ± 0.37	0.765 ± 0.24	0.647 ± 0.22†	0.501 ± 0.09*	0.435 ± 0.09*
Li (µg/mL)	5.416 ± 1.46	4.741 ± 1.22	5.058 ± 1.35	5.394 ± 1.312	5.350 ± 1.35

Compared with the control, *p<0.01, †p<0.05, ‡p<0.001,

A positive correlation was observed between the oral F concentrations and the serum Co levels, whereas negative correlations were present with the serum levels of Mo, Cr, and Mn, but no correlation could be detected with the serum level of Li (Table 3).

Table 3. Pearson correlation test results (r) and their p values between dosage of oral F and serum Co, Mo, Cr, Mn, and Li levels

	F – Co	F – Mo	F – Cr	F – Mn	F – Li
Correlation Value (r)	0.488‡	-0.790‡	-0.422*	-0.653‡	0.057

*p<0.01, ‡p<0.001.

The effect of F supplementation in drinking water on the serum levels of Co, Mo, Cr and Mn, and the results of regression analyses are given in Table 4.

Table 4. Regression analyses for F in the drinking water and the serum Co, Mo, Cr, and Mn levels

Response	Predictor	Regression Equation
Co	Oral F concentration	Co (ng/mL) = 0.317 + 0.00199 F concentration (mg/L) ‡
Mo		Mo (ng/mL) = 0.317 + 0.00199 F concentration (mg/L) ‡
Cr		Cr (ng/mL) = 0.386 – 0.00103 F concentration (mg/L)*
Mn		Mn (ng/mL) = 0.925 – 0.0132 F concentration (mg/L) ‡

*p<0.01, ‡p<0.001.

DISCUSSION

Various investigations have reported effects of F intoxication that caused deficiency or decreases in serum Ca, Mg, K, Cu, Zn, and Se, [15-22,24] but, to the best of our knowledge, there has been no study on the interaction of F and serum levels of Co, Mo, Cr, Mn, and Li.

In the present study with mice, when F intake levels were increased, the serum level of Co increased and the serum levels of Mo, Cr, and Mn decreased. The serum level of Li, however, was essentially unaffected (Tables 2–4). F has a strong affinity for cations, especially polyvalent ones,[10-11] and although Co and Li are cations like the others, F did not decrease their serum levels.

In connection with the F-induced decrease in serum Mn observed here, Birkner et al.[25] reported that F has an inhibitory effect on Mn-dependant enzyme activity. The failure of F to decrease the serum levels of Co and Li may be due to the fact that the F level in the drinking water of the mice was not sufficient to show significant binding of serum Co and Li, except for the Co level in the 40 mg F/L group. This may be attributed to renal insufficiency during F intoxication.[2,26,27] Probably higher F concentrations will be effective in binding serum Co and Li levels. Although serum Co levels increased in the 40 mg F/L group, they and the Li serum levels were within physiological ranges.[9] Mo, Cr, and Mn are also crucial minerals for body functions,[8,9] and decrease in the serum levels of these minerals must be considered in connection with F ingestion.

Overall, serum levels of Co, Mo, Cr, and Mn in mice were affected by F supplementation, but the serum level of Li was not. Especially noteworthy was the decrease in Mo, Cr, and Mn, which might affect various crucial metabolic pathways in mammals, including humans.

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