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Original Article

The determination of urine protein and creatinine concentrations in the urine of HIGA mice and BALB/c mice after subacute administration of fluoride via their drinking water

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Summary

Fluoride (F) widely exists in nature and is contained in food and water. One of the target organs of F is the kidney. High IgA (HIGA) mice have been used as a model of IgA nephropathy. The objective of this study was to get information for the alterations in the renal function of HIGA mice exposed to F. The HIGA and BALB/c mice were exposed to F at 0, 50, 100, and 150 ppm in their drinking water for 4 weeks. For the protein levels in the urine, the mean of the 100-ppm group of HIGA mice after 3 weeks from the beginning of the exposure was significantly higher than those in the 0- and 50-ppm groups. For BALB/c mice, the mean protein level in the 100-ppm group was significantly higher than those in the 0- and 50-ppm groups after 2 weeks. The mean creatinine in the urine in the 50-, 100-, and 150-ppm groups of HIGA mice were significantly lower than that in the 0-ppm group after 4 weeks. For BALB/c mice, the mean creatinine in the 50-, 100-, and 150-ppm groups were significantly lower than that in the 0-ppm group after 2 weeks. The exposure to F at 100 ppm may induce temporal toxicity to the kidneys of mice, but no differences of toxic effects of F between the 11- to 12-week-old HIGA and BALB/c mice were observed.

Introduction

Fluoride (F) widely exists in nature and is contained in food. While, it is also known as an environmental pollutant. The contamination of ground water by F have been reported in China[1)] and India[2,3)]. Excessive F intake over time results in dental and osteofluorosis. Many inhabitants who drink well water with high concentrations (8 ppm and over) of F suffer from endemic osteofluorosis[2,4)]. In Japan, since the levels of F in the ground water is not high as 8 ppm[5)], osteofluorosis rarely occurs. The levels of F in hot springs were high in some areas in Japan, and it is recommended not to drink the water from such hot springs[6)]. While, although the preventive effect of F on dental caries at around 1 ppm in the drinking water has been suggested[5)], to our knowledge, there have been no reports on the effects of F deficiency. Therefore, as a nutrient, it is still controversial whether or not F is an essential element. For the possible use of F as a nutrient, the toxicities of F must be elucidated.

In addition to the toxic effects of F on teeth and bones, the kidney is a target organ of F[5)]. Manocha et al. reported the renal toxicity of F in monkeys perfused with the solution contained F at 1.5 mM[7)]. In addition, greater accumulation of F occurred in Institute of Cancer Research (ICR)-derived glomerulonephritis mice (ICGN) which have impaired renal function and affected them more seriously in our previous studies[8,9)]. In those studies, all the ICGN mice exposed to 150 ppm F in their drinking water died within 4 weeks. The mean values of blood urea nitrogen (BUN) and serum creatinine in the ICGN mice exposed to F at 150 ppm in their drinking water were significantly higher than those in the control. The serum F was significantly higher in the ICGN mice expose to F. In the patho-

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logical findings, deterioration due to glomerular sclerosis was observed [9].

IgA nephritis is the most common chronic glomerulonephritis among Japanese [10]. More than 20% of the adult patients suffered IgA nephritis, and among the children, more than 30% of the patients had IgA nephritis. Although, if treated in its early stages, the prognosis of IgA nephritis is good, patients who are untreated in the advanced stage progress to renal hypertension and at the end stage, renal insufficiency. About 30% of patients with IgA nephritis develop renal insufficiency within 15 to 20 years, and 5% to 10% of them develop renal insufficiency within 5 years [10, 11]. It is of interest whether or not the exposure to F via drinking water exacerbates nephropathy of patients with IgA nephritis.

High IgA (HIGA) mice, a mutant of ddY mice, develop IgA nephropathy spontaneously [11, 12]. HIGA mice have often been used as a model of IgA nephropathy. It is of interest whether the administration of F exacerbates the kidney function of HIGA mice or not. The urine of each mouse is relatively easy to be sampled by using a metabolic cage.

In this study, we evaluated creatinine and protein concentrations in the urine of HIGA and BALB/c mice that were administered F via their drinking water. The objective of this study was to get basic information for the alterations in the renal function of the HIGA mice exposed to F via their drinking water. The information may be useful to elucidate the effects of F on patients with IgA nephropathy and to consider the possible use of F as a nutrient.

Materials and Methods

Experimental mice and F exposure

The male HIGA mice, 11 to 12 weeks of age, were obtained from Japan SLC, Inc. (Hamamatsu, Japan). BALB/c mice (Japan SLC) were used as a normal kidney function control. The male HIGA and BALB/c mice were exposed to F at concentrations of 0, 50, 100, or 150 ppm (n = 5/group) in their drinking water for 4 weeks. There were no significant differences in body weight or BUN among the groups of HIGA or BALB/c mice before the exposure. Mice drank the water ad libitum. The care and treatment of the mice were in accordance with the guidelines established by the Animal Experimentation and Ethics Committee of Kitasato University School of Medicine and were approved by the committee.

Body weight and the sampling of urine by a metabolic cage

The body weight of each mouse was checked twice a week. The urine of each mouse was sampled by using a metabolic cage. We only sampled the urine of mice, once a week, using metabolic cages, because stress to mice in metabolic cages has been reported [13]. Each mouse was housed in a metabolic cage for 24 hours once a week. To avoid pieces of rodent chow and feces falling into the urine, a stainless steel funnel was put into the collecting tube at the bottom of the metabolism cage.

Determinations of protein and creatinine levels in the urine

The protein level in the urine was determined by the Bradford assay, Quick Start (Bio-RAD, Tokyo, Japan). The standard solutions of protein or the samples (triplicate for each sample) were added to 200 μl of 5-times diluted Bradford reagent in wells of a 96-well microplate. The microplate was incubated for 5 minutes at room temperature. The absorbance at λ 595 nm was measured using a Power Scan TH (BioTAK, Tokyo) and the protein concentrations were determined by a standard curve. The creatinine concentrations in the urine were determined by the Enzyme method by SRL (Special Reference Laboratories, Tokyo) [14, 15].

Statistical analyses

The mean values of the final body weight, protein, and creatinine in the urine of each group were calculated and compared by one-way ANOVA (analysis of variance) followed by Student-Newman-Keuls test. Statview J-5.0 software (SAS Institute, Cary, NC, U.S.A) was used for all statistical analyses. The level of significance was $P < 0.05$.

Results

The final body weights of the HIGA and BALB/c mice are shown in Table 1. There were no significant differences in the final body weights among the 0 ppm- and F-ex-

Table 1. Mean values of body weight of HIGA and BALB/c mice exposed to fluoride in the drinking water the final.

	HIGA	P-value [a]		BALB/c	P-value [a]
0 ppm	43.52 ± 1.31 [b]	0.487	0 ppm	27.43 ± 0.58 [b]	0.183
50 ppm	46.46 ± 1.80		50 ppm	28.25 ± 0.30	
100 ppm	45.53 ± 0.97		100 ppm	28.33 ± 0.51	
150 ppm	44.39 ± 1.38		150 ppm	27.15 ± 0.26	

[a] P - value by ANOVA, [b] Means ± standard errors are indicated.

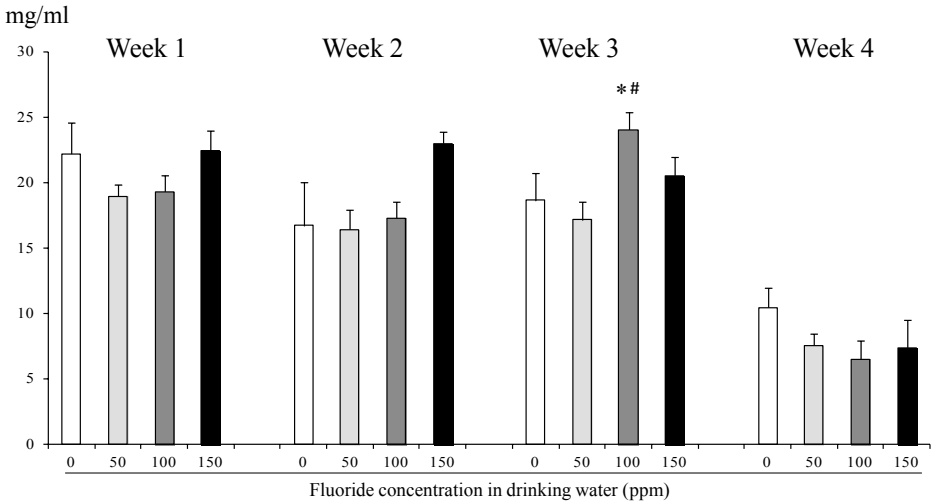

Fig. 1 The protein levels in the urine of HIGA mice exposed to fluoride in their drinking water.

※ Mean values and standard errors are indicated (n=5/group). P=0.052 for week 1, P=0.082 for week 2, P=0.009 for week 3, P=0.333 for week 4 for by ANOVA. *P<0.05 (vs 0 ppm) #P<0.05 (vs 50 ppm) by Student-Newman-Keuls test.

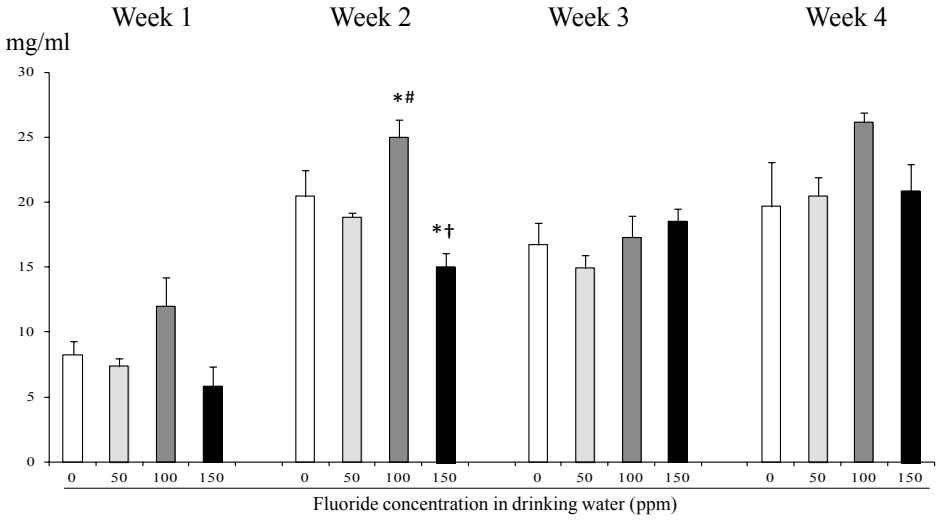

Fig. 2 The protein levels in the urine of BALB/c mice exposed to fluoride in their drinking water.

※ Mean values and standard errors are indicated (n=5/group). P=0.052 for week 1, P=0.0007 for week 2, P=0.336 for week 3, P=0.174 for week 4 by ANOVA. *P<0.05 (vs 0 ppm), # P<0.05 (vs 50 ppm), † P<0.05 (vs 100 ppm) by Student-Newman-Keuls test.

posed HIGA mice or the 0 ppm and F-exposed BALB/c mice.

Figure 1 shows the protein levels in the urine of HIGA mice exposed to F in their drinking water. After 3 weeks from the beginning of the exposure, the mean value of the protein level in the urine in the 100-ppm group was significantly higher than those in the 0- and 50-ppm groups. Figure 2 shows the protein levels in the urine of BALB/c mice exposed to F in their drinking water. After 2 weeks from the beginning of the exposure, the mean value of the protein level in the urine in the 100-ppm group was significantly higher than those in the 0- and 50-ppm groups. The mean protein level in the urine in the 150-ppm group was significantly lower than that in the 0- and 100-ppm group.

Figure 3 shows the creatinine levels in the urine of HIGA mice exposed to F in their drinking water. After 4 weeks from the beginning of the exposure, the mean values of the creatinine levels in the 50-, 100-, and 150-ppm groups were significantly lower than those in the 0-ppm groups. Figure 4 shows the creatinine levels in the urine of BALB/c mice exposed to F in their drinking water. After 2 weeks from the beginning of the exposure, the mean values of the creatinine levels in the 50-, 100-, and 150-ppm groups were significantly lower than those in the 0-ppm groups.

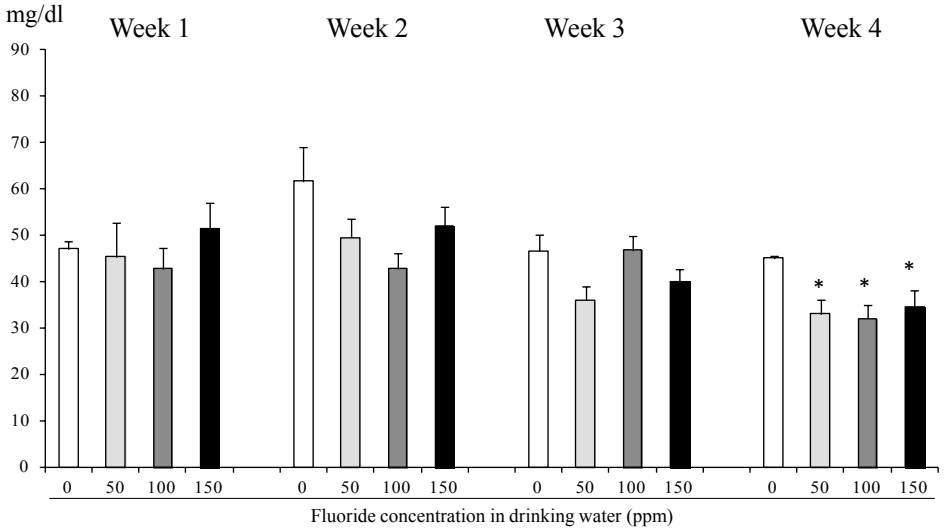

Fig. 3 The creatinine levels in the urine of HIGA mice exposed to fluoride in their drinking water.

※ Mean values and standard errors are indicated (n=5/group). P=0.712 for week 1, P=0.096 for week 2, P=0.078 for week 3, P=0.018 for week 4 by ANOVA. *P<0.05 (vs 0 ppm), by Student-Newman-Keuls test.

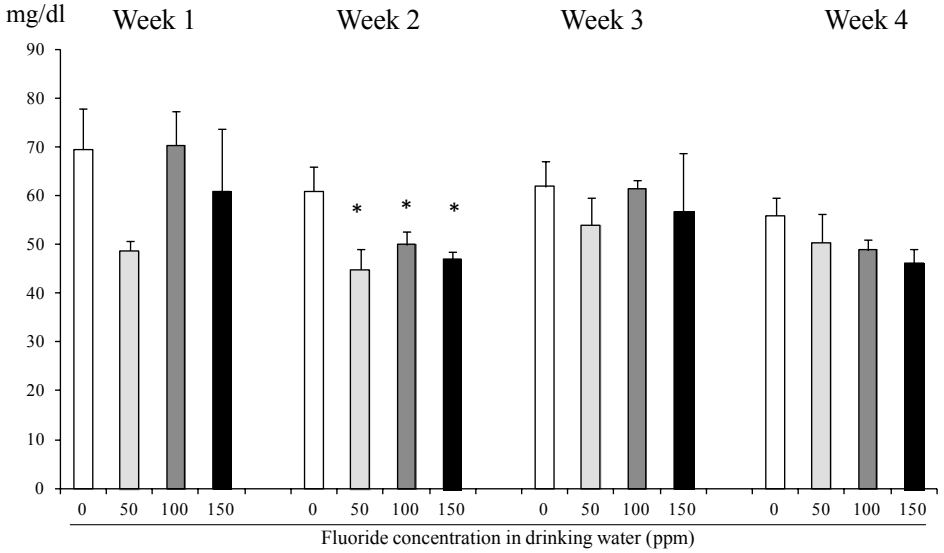

Fig. 4 The creatinine levels in the urine of BALB/c mice exposed to fluoride in their drinking water.

※ Mean values and standard errors are indicated (n=5/group). P=0.286 for week 1, P=0.027 for week 2, P=0.833 for week 3, P=0.376 for week 4 by ANOVA. *P<0.05 (vs 0 ppm), by Student-Newman-Keuls test.

Discussion

It is still controversial whether or not F is an essential element. It is also known as an environmental pollutant and the contamination of ground water by F has been reported in China [1] and India [2, 3]. Drinking water containing F at 8 ppm and over induced osteofluorosis. One of the target internal organs of F is the kidney. Since F is excreted from the kidney, the toxic effects of F may be enhanced when the renal function of the patients or experimental animals are impaired. It is also of interest whether or not the toxic effects of F by the exposure via drinking water deteriorate nephropathy of patients with IgA nephritis. Therefore, the present study was focused on the toxicities of F on the kidneys of HIGA mice. We chose HIGA mice because they are a well-known animal model of IgA nephritis. The F concentrations in the drinking water were decided following our previous studies for ICGN mice. The level of 150 ppm is approximately 10 times higher compared to the actual highest concentrations in well-water in India and China.

There were no significant differences for the mean values of the final body weights and viabilities among the HIGA mice aged 11 to 12 weeks exposed to F in their drinking water at 0, 50, 100, or 150 ppm. In our previous studies, all the ICGN mice administered F at 150 ppm died

within 4 weeks[8, 9]. The body weights of the ICGN mice exposed to F showed marked decreases. Some of the ICGN mice exposed to F at 100 ppm also died within 4 weeks. The difference between HIGA mice in this study and ICGN mice in previous studies may be due to the advances in the deteriorations of renal function. Actually, for mice with normal function, the BALB/c mice administered F at 125 ppm[16] and the ICR mice administered F at 150 ppm[8, 9] did not die after 4 weeks of the exposures and showed no differences in body weights compared with the respective control.

For the protein levels in the urine of the HIGA and BALB/c mice, the mean values of the protein levels in the 100-ppm group of HIGA mice after 3 weeks from the beginning of the exposure was significantly higher than those in the 0- and 50-ppm groups. For BALB/c mice, after 2 weeks from the beginning of the exposure, the mean value of the protein level in the 100-ppm group was significantly higher than those in the 0- and 50-ppm groups. That in the 150-ppm group, however, was significantly lower than that in the 0- and 100-ppm group. The relationship between the protein level in the urine and the dose of F was not dose-dependent, and the higher protein concentrations in the urine in the 100-ppm groups of the HIGA or BALB/c mice were observed temporarily. Therefore, the effects of F on the urine protein levels were not clear, which are in accordance with the results of ICR mice in our previous studies[8, 9]. The higher protein levels in the urine of the 100-ppm groups were observed in both the HIGA and BALB/c mice, suggesting that the renal functions between the HIGA and BALB/c mice were not different at those ages. After 4 weeks from the beginning of the exposure, the mean values of the creatinine levels in the 50-, 100-, and 150-ppm groups of the HIGA mice were significantly lower than those in the 0-ppm groups. For the BALB/c mice after 2 weeks from the beginning of the exposures, the mean values of the creatinine levels in the urine in the 50-, 100-, and 150-ppm groups were significantly lower than those in the 0-ppm groups. The significance of these observations was not clearly elucidated. For creatinine, the levels in the serum should be determined in the further studies.

The differences of toxic effects of F between the HIGA and BALB/c mice were not clearly observed in this study. The IgA nephritis of HIGA mice may not advance further in 11 to 12 weeks. For the model of patients with IgA nephritis, older HIGA mice, in which IgA nephritis is most likely to be advanced, should be used. For HIGA mice, it is not clear whether the increase in the urine protein level at 1 week after exposure and the decrease after 4 weeks are due to a toxic mechanism or by chance. Also, the changes in urine proteins in BALB/c mice over the course of time could not be elucidated, therefore, further studies are warranted.

In conclusion, the exposure to F at 100 ppm in drinking water could induce toxic effects to the kidneys of mice, and the differences of the toxic effects of F between the HIGA and BALB/c mice were not clearly observed by the exposure to mice of 11 or 12 weeks of age. Regarding the possible use of F as a nutrient, it should be noted that the exposure to F at 100 ppm in drinking water could induce at least temporal toxic effects to kidneys in mice with normal kidney function.

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