THE EFFECTS OF FLUORIDE ON THE BONES AND TEETH FROM ICR-DERIVED GLOMERULONEPHRITIS (ICGN) MICE AND ICR MICE AFTER SUBACUTE EXPOSURE

Mayuko Hosokawa,[a] Chiemi Sugaya,[b] Masashi Tsunoda,[b] Yukio Kodama,[c] Yoshiko Sugita-Konishi,[d] Hisayoshi Ohta,[e] Kazuhito Yokoyama[a]

Tokyo, Japan

ABSTRACT: Dental fluorosis and osteofluorosis from using drinking water contaminated with the fluoride ion (F) have been reported from many countries including the People's Republic of China and India. Because fluoride is excreted by the kidney and the toxic effects of F are more severe when renal failure is present, Imprinting control region (ICR)-derived glomerulonephritis (ICGN) mice, which have been used as a model of renal failure, could be a useful model for osteofluorosis. In the present study, ICGN mice and ICR mice were administered F at 0, 25, 50, 100, and 150 ppm in their drinking water for 4 weeks. The femurs of the mice were photographed and analyzed by microdensitometry. The teeth were stained with hematoxylin and eosin. While no significant differences in any bone indexes were found in the ICGN groups, the mean values for bone mineral content and bone mineral density of the left femur from the male ICR 150 ppm group were significantly higher than those of the control group mice ($p<0.001$). Partial heterogeneous calcification of the tooth dentine was observed in the ICGN mice exposed to 150 ppm. ICGN mice may be used as a model for dental fluorosis. However, ICR mice, particularly males, with an exposure period of 2–4 mo, may be a better animal model for osteofluorosis.

Keywords: Bones; Fluoride; ICGN mice; ICR mice; Subacute administration; Teeth.

INTRODUCTION

Instances of contamination of groundwater by the fluoride ion (F) have been reported in many countries including the People's Republic of China and India. In several developed countries, such as the United States of America, water fluoridation has been introduced for the prevention of caries.[1] The excess intake of F induces enamel hypoplasia and deficient calcification in teeth. Moreover, F at 4 ppm induces dental fluorosis, also called mottled teeth, and F at 8 ppm induces systemic fluorosis.[2]

The main target of systemic fluorosis, also known as skeletal fluorosis, is bone.[3] A high amount of F in the drinking water induces skeletal fluorosis after extended exposure. Endemic skeletal fluorosis has been reported in districts with high concentrations of F in the drinking water in developing countries such as the People's Republic of China[4] and India.[5] In addition to the known pathophysiology of skeletal fluorosis involving the synthesis of fluoroapatite in the bone, other

[a]Department of Epidemiology and Environmental Health, Juntendo University Faculty of Medicine, Tokyo, Japan; [b]Department of Hygiene, Kitasato University School of Medicine, Kanagawa, Japan; [c]National Institute of Sciences, Setagaya, Tokyo, Japan; [d]Department of Food and Life Sciences, School of Life and Environmental Sciences, Azabu University, Kanagawa, Japan; [e]Department of Environmental, Occupational Health and Toxicology, Kitasato University School of Allied Health Sciences, Kanagawa, Japan; *For correspondence: Dr Masashi Tsunoda. Department of Hygiene, Kitasato University School of Medicine, 1-15-1 Kitasato, Minami-ku, Sagamihara, Kanagawa 252-0374, Japan; E-mail: mtsunoda@med.kitasato-u.ac.jp; phone: 81 42 778 9311; fax: 81 42 778 9257.

mechanisms have been proposed such as F promoting bone formation by an increase of osteoblasts.[3] To elucidate skeletal fluorosis, establishing an animal model for skeletal fluorosis is helpful. However, the alteration in bone by F requires extended exposure, so that it is not easy to establish an animal model of skeletal fluorosis.

F is excreted in the urine through the kidneys.[6] If a patient's renal function is deteriorated, F would increase in the blood and accumulate in the bones. Enhanced toxic effects on body weight and the kidneys were observed in previous studies using experimental mice with renal insufficiency.[7,8] In studies on the effects of F on the bones and teeth of normal rats and mice, by the National Institutes of Health (NIH), USA, which detected differences from the controls, the observation period was 6 mo.[9] In a previous study, Turner et al.[10] determined the effects of F on skeletal fragility and mineralization in renal-deficient animals. They created renal deficiency by surgical resection of 80% or 88% of the total renal mass of the Sprague-Dawley rats.[10,11] The rats were exposed to F via drinking water at 0, 5, 15, and 50 ppm for 6 mo. The vertebral osteoid volume was increased in the rats with renal deficiency who received 15 or 50 ppm F. However, the resection of 80% or 88% of the total renal mass produced a different situation from that of the patients with renal insufficiency.

We hypothesized that the alteration in bone induced by F could be detected in model animals with renal insufficiency after a relatively short exposure. For an animal model with renal insufficiency, imprinting control region (ICR)-derived glomerulonephritis (ICGN) mice, which spontaneously develop glomerulonephritis, have been used.[12] To establish an adequate animal model for skeletal fluorosis, it is necessary to evaluate the effects of F on the bones of ICGN mice as well as ICR mice which have normal kidney function. By using such an evaluation as a benchmark, whether or not osteofluorosis has developed in mice with renal insufficiency could be elucidated.

An animal model for the development of skeletal fluorosis after F exposure could be evaluated by microdensitometry, which is a relatively simple method for evaluating alterations in bone.[11] Therefore, we used microdensitometry to evaluate the femurs from the ICGN and ICR mice exposed to F.

Moreover, the alterations in teeth caused by F might be observed earlier in this animal model. In the study by the NTP (National Toxicology Program),[9] the detection of a difference in the teeth in the F-exposed animals from the controls also took 6 mo. Thus, evaluating the pathological effects of F on teeth in the ICGN mice would be worthwhile.

In the present study, we administered F to ICGN and ICR mice via their drinking water for 1 mo. The objective of the study was to use microdensitometry to examine the pathology of the bones and teeth of mice with renal insufficiency that were exposed to increasing concentrations of F.

MATERIALS AND METHODS

ICGN mice with a level of blood urea nitrogen (BUN) in the serum of 27.0 mg/dL, originating from mutant ICR mice, were obtained from the National Institute

of Health and Sciences, Tokyo, Japan. ICR mice with normal kidney function were used as a control group (Oriental Yeast Co., Ltd., Tokyo, Japan).

The male and female ICGN mice were exposed to F at 0, 25, 50, and 100 ppm (n=5 for each male group and n=4 for each female group) and 150 ppm (n=5 for males and n=7 for females) in their drinking water for 4 wk. The male and female ICR mice were exposed to F at 0 (n=10 for males and n=7 for females) and 150 ppm (n=11 for males and n=7 for females) in their drinking water for 4 weeks. For the adjustment of the F concentrations in their drinking water, NaF was dissolved in the tap water which had a F concentration of <0.8 ppm. The mice were fed commercial rodent chow, and housed in a temperature-controlled (25ºC) animal facility, with automatic light/dark cycles of 14/10 hr. When mice died before the the end of the 4-week period, their femurs were sampled at that time. At the end of the 4-week observation period, the mice that were still alive were euthanized and their femurs and teeth sampled.

The femurs were examined by microdensitometry[13] They were photographed with a standard aluminum scale ruler with Softex x-ray equipment. The pattern of optical bone density was compared with the optical density of a standard aluminum scale in the photos and evaluated. The cross sectional pattern at the middle line of the bone was transformed as ΣGS by comparison with the optical density of the aluminum scale, in which the optical density appeared in a step-wedge fashion.[14] The bone mineral content, the bone mineral density, and the cortical thickness of each bone were calculated as indexes. The formulae for the indexes were:

Medullary bone width = d

Bone width = D

Bone mineral content = ΣGS

Bone mineral density was evaluated by two indexes:

$$\text{Bone mineral density} = \frac{\Sigma GS}{D}$$

$$\text{Bone mineral density} = \frac{\Sigma GS}{D^2}$$

$$\text{Critical thickness index} = \frac{(D - d)}{D}$$

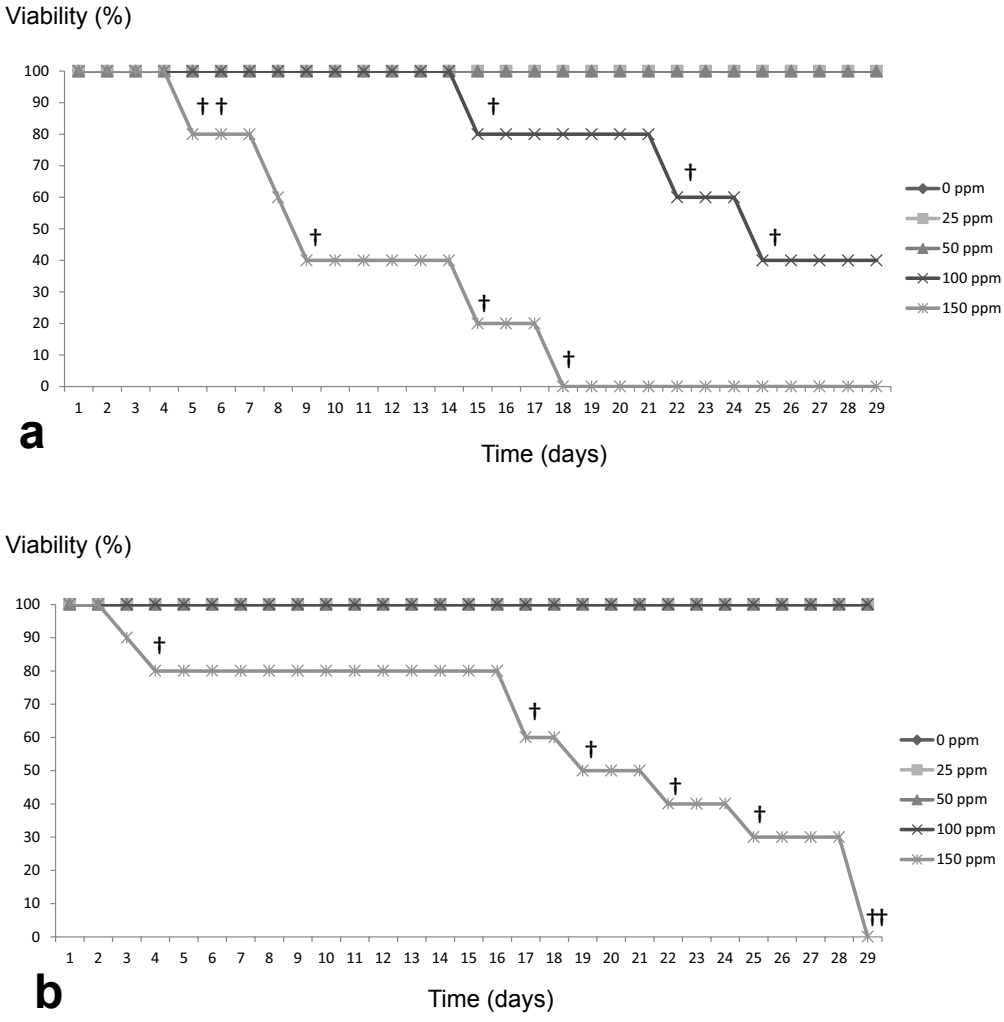
The teeth from the ICGN and ICR mice were decalcified in EDTA (ethylenediaminetetraacetic acid) solution, routinely processed, and stained with hematoxylin and eosin (HE). After paraffin embedding, the specimens were sliced and observed with a light microscope.

The deaths of mice during the administration period were recorded, and the viability of the mice calculated. Their body weights after the observation period were compared, with the exception of those in the 150 ppm group which were not included because of their early deaths. The mean values of the indexes of

microdensitometry for femurs in each group were calculated. The mean values among the ICGN groups were compared by one-way ANOVA (analysis of variance) using Statview 5.02 v (SAS, Cary, CA, USA), followed by the Student-Newman-Keuls test as a post hoc test (significance level, $p<0.05$). The mean values between the ICR groups were compared with the *t* test or the Mann Whitney U test.

RESULTS

The viability of the ICGN mice exposed to F at 0, 25, 50, 100, and 150 ppm in their drinking for water for 1 mo is shown in Figure 1(a) for males and Figure 1(b) for females.



Figures 1a and 1b. Viabilities of ICGN mice of male (a) and female (b) mice exposed to 0, 25, 50, and 100 ppm F in their drinking water for 1 mo.

All the ICGN mice exposed to F at 150 ppm died before the end of the 4-week observation period. In addition, 3 of 7 male ICGN mice exposed to F at 100 ppm

died during the observation period. However, the female ICGN mice exposed to F at 100 ppm did not die during the 4-week period. No ICR mice exposed to F at 150 ppm died during the observation period.

The mean values of body weight for the ICGN and ICR mice at the end of the observation period are shown in Table 1.

Table 1. Mean values of the weights of (a) ICGN mice and (b) ICR mice exposed to F at the end of the observation period

(a) ICGN mice		
F concentration (ppm)	Male	Female
0	29.0±0.94	21.3±0.74
25	29.9±2.85	21.3±0.92
50	28.6±1.17	20.3±0.70
100	27.0±1.65	19.3±2.12
(b) ICR mice		
F concentration (ppm)	Male	Female
0	40.3±1.09	38.6±0.90
150	40.1±1.30	39.7±1.01

The ICGN mice exposed to F at 150 ppm showed a marked decrease in body weights at their death. Although not significantly different, the mean body weights in the male and female ICGN mice exposed to F at 100 ppm were lower than those in the control group. There were no significant differences in the body weights between the control and the 150-ppm-exposed ICR mice for either the male or the female mice.

The results of the microdensitometry examination of the femurs from the ICGN mice exposed to F are summarized in Table 2. There were no significant differences in any of the bone indexes among the ICGN groups. The microdensitometry results for the femurs from the ICR mice are summarized in Table 3. The mean values of ΣGS, ΣGS/D, and $\Sigma GS/D^2$ of the left femurs from male ICR mice in the 150 ppm group were significantly higher than those from the control group. The mean value of $\Sigma GS/D^2$ of the right femurs from the male ICR mice in the 150 ppm group was significantly higher than that from the control group. The mean value of ΣGS of the right femurs from the male ICR mice in the 150 ppm group tended to be higher than that from the control group ($p=0.086$). The mean value of ΣGS/D of the right femurs was also non-significantly higher than that from the control group. For female ICR mice, the mean value of ΣGS of the left femur from the 150 ppm group tended to be higher than that from the control group ($p=0.098$). The mean value of $\Sigma GS/D^2$ of the right femur from the 150 ppm group tended to be higher than that from the control group ($p=0.071$).

Table 2. The results of microdensitometry of femurs from ICGN mice exposed to fluoride (Values are mean±SE, R=right, L=left)

(a) ΣGS (Bone mineral content)

F concentration (ppm)	Male		Female	
	ΣGS (R)	ΣGS (L)	ΣGS (R)	ΣGS (L)
0	1.00±0.07	1.12±0.05	1.00±0.07	1.12±0.05
25	1.06±0.06	1.15±0.09	1.06±0.06	1.15±0.09
50	1.08±0.08	1.15±0.07	1.08±0.08	1.15±0.07
100	1.09±0.09	1.16±0.10	1.09±0.09	1.16±0.10
150	1.15±0.04	1.19±0.06	1.15±0.04	1.19±0.06

(b) ΣGS/D (Bone mineral density)

F concentration (ppm)	Male		Female	
	ΣGS/D (R)	ΣGS/D (L)	ΣGS/D (R)	ΣGS/D(L)
0	0.53±0.04	0.53±0.03	0.53±0.04	0.53±0.06
25	0.55±0.05	0.56±0.04	0.55±0.05	0.56±0.04
50	0.57±0.04	0.59±0.02	0.57±0.04	0.59±0.03
100	0.56±0.05	0.60±0.30	0.57±0.05	0.60±0.03
150	0.54±0.05	0.63±0.01	0.54±0.05	0.63±0.03

(c) $\Sigma GS/D^2$ (Bone mineral density)

F concentration (ppm)	Male		Female	
	$\Sigma GS/D^2$ (R)	$\Sigma GS/D^2$ (L)	$\Sigma GS/D^2$ (R)	$\Sigma GS/D^2$ (L)
0	0.26±0.03	0.32±0.02	0.29±0.03	0.32±0.02
25	0.29±0.04	0.28±0.03	0.29±0.04	0.28±0.03
50	0.30±0.24	0.31±0.02	0.30±0.02	0.31±0.02
100	0.28±0.03	0.31±0.02	0.28±0.03	0.31±0.02
150	0.29±0.01	0.34±0.03	0.29±0.01	0.34±0.03

(d) D (Bone width)

F concentration (ppm)	Male		Female	
	D (R)	D (L)	D (R)	D (L)
0	0.35±0.01	0.68±0.28	0.35±0.14	0.68±0.28
25	0.40±0.03	0.40±0.16	0.40±0.31	0.40±0.16
50	0.36±0.02	0.38±0.16	0.36±0.02	0.38±0.16
100	0.36±0.02	0.36±0.04	0.36±0.23	0.36±0.04
150	0.58±0.04	0.37±0.02	0.58±0.17	0.37±0.02

(e) d (Medullary bone width)

F concentration (ppm)	Male		Female	
	d (R)	d (L)	d (R)	d (L)
0	1.62±0.12	1.52±0.37	1.62±0.12	1.52±0.37
25	1.95±0.32	2.16±0.23	1.95±0.32	2.16±0.23
50	1.71±0.09	1.83±0.16	1.71±0.09	1.83±0.16
100	1.82±0.15	1.71±0.20	1.82±0.15	1.71±0.20
150	1.60±0.36	1.73±0.18	1.60±0.36	1.73±0.18

Table 3. The results of microdensitometry of femurs from ICR mice exposed to fluoride (Values are mean±SE, R=right, L=left)

(a) ΣGS (Bone mineral content)

F concentration (ppm)	Male		Female	
	ΣGS (R)	ΣGS (L)	ΣGS (R)	ΣGS (L)
0	1.19±0.20	1.01±0.07	1.71±0.14	1.73±0.11
150	1.60±0.12	1.70±0.13*	1.94±0.98	1.97±0.08

(b) ΣGS/D (Bone mineral density)

F concentration (ppm)	Male		Female	
	ΣGS/D (R)	ΣGS/D (L)	ΣGS/D (R)	ΣGS/D(L)
0	0.50±0.06	0.47±0.04	0.68±0.06	0.69±0.05
150	0.66±0.07	0.79±0.05*	0.85±0.56	0.76±0.09

(c) $\Sigma GS/D^2$ (Bone mineral density)

F concentration (ppm)	Male		Female	
	$\Sigma GS/D^2$ (R)	$\Sigma GS/D^2$ (L)	$\Sigma GS/D^2$ (R)	$\Sigma GS/D^2$ (L)
0	0.24±0.02	0.21±0.02	0.31±0.19	0.27±0.03
150	0.35±0.03*	0.36±0.02*	0.37±0.04	0.38±0.03

(d) D (Bone width)

F concentration (ppm)	Male		Female	
	D (R)	D (L)	D (R)	D (L)
0	0.34±0.03	0.34±0.02	0.31±0.03	0.36±0.02
150	0.72±0.24	0.37±0.02	0.36±0.02	1.00±0.59

(e) d (Medullary bone width)

F concentration (ppm)	Male		Female	
	d (R)	d (L)	d (R)	d (L)
0	2.20±0.38	2.18±0.14	2.23±0.20	3.02±0.26
150	1.82±0.29	2.34±0.26	2.54±0.29	2.54±0.43

Using the t test, compared to the control group: *p<0.001.

The HE-stained samples for the pathological examination of the enamel of the teeth from the ICGN and ICR mice exposed to F at 150 ppm in their drinking water are shown in Figure 2. For the ICGN mice, partial heterogeneous

calcification occurred in the dentin. For the ICR mice, heterogeneous structures of residual organic components were observed in the enamel.

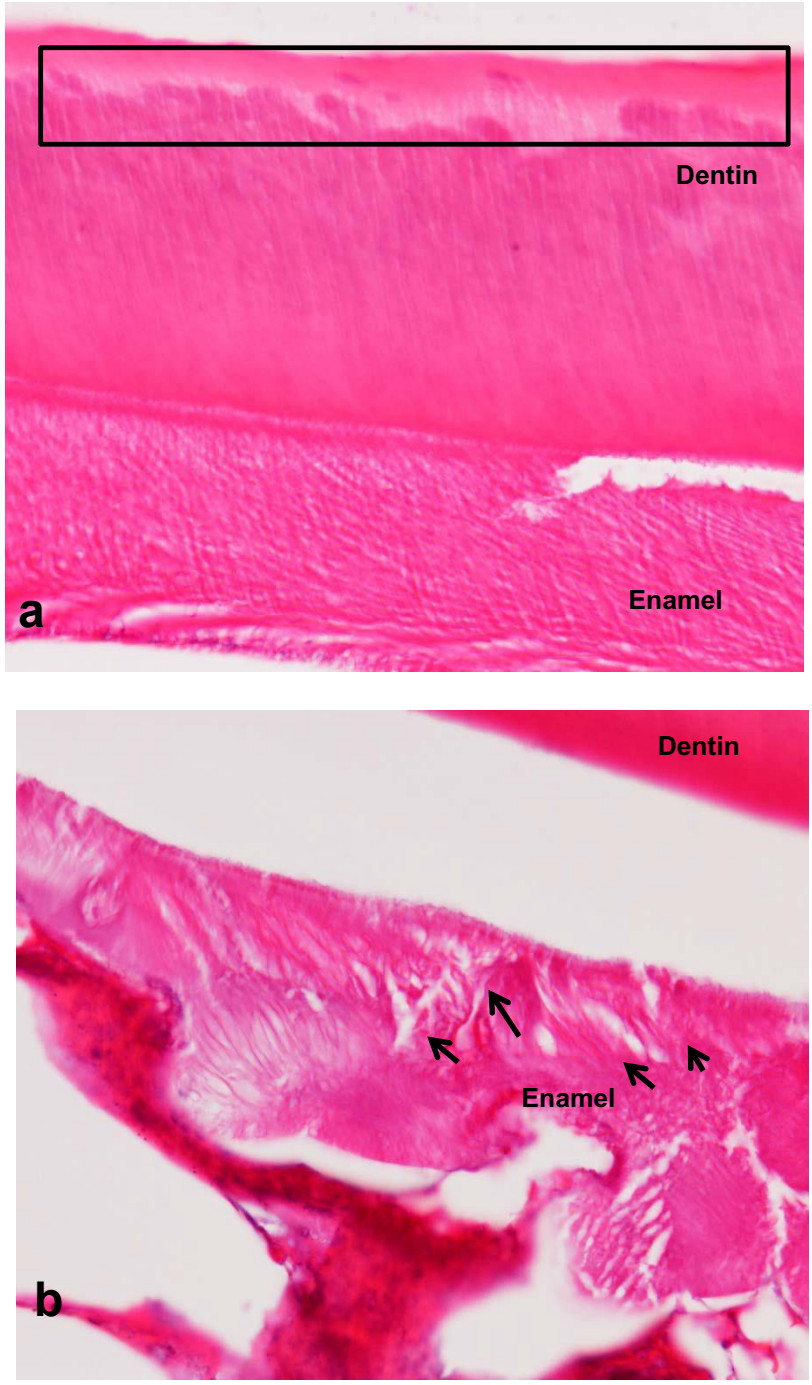


Figure 2. The tooth enamel from ICGN and ICR mice exposed to F at 150 ppm in their drinking water (hematoxylin and eosin stain, × 40). (a) ICGN mice and (b) ICR mice. For the ICGN mice, partial heterogeneous calcification occurred in the dentin (rectangle). For the ICR mice, heterogeneous structures of residual organic components were observed in the tooth enamel.

DISCUSSION

Skeletal fluorosis and dental fluorosis caused by F intake from drinking water have occurred frequently in humans in India[5,15] and the People's Republic of China.[4,16,17] However, all the factors that cause these F-induced alterations in bone and teeth have not yet been discovered. We hypothesized that impaired kidney function is one of the factors contributing to the F-induced deterioration in the structure of bone and teeth. To elucidate the role of the kidney in the alteration in bone and teeth induced by F, the development of an adequate animal model is needed. Turner et al.[10] created renal insufficiency in rats by nephrectomy. They evaluated F-induced toxicity by assessing skeletal fragility and osteoid volume in the rats' vertebrae. For an animal model, a relatively simple method for evaluation is helpful. Microdensitometry is a simple method that is used in humans to evaluate bone mineral content, bone mineral density, cortical thickness index, and bone width.[13] It can also be used in animals. The objective of the present study was to determine whether or not the effects of F on bones and teeth were exacerbated in mice with impaired renal function by evaluating the femurs by microdensitometry and the teeth by examining hematoxylin and eosin stained sections for pathological changes.

Although we did not determine the F concentrations in the rats' serum, in a previous study,[7] the mean F level in the serum of the ICGN mice exposed to F at 100 ppm for 4 weeks was more than 2.0 mg/L. The serum level of F in the ICR mice exposed to 150 ppm in the present study was 0.50 mg/L. Because the protocol in this study was the same as that in the previous study, the F concentrations in the rats' serum were considered to be similar. In the study by Turner et al.,[10] the F in the plasma from the rats with renal deficiency exposed to 50 ppm of F was 1.5 mg/L. For the control rats, the F in plasma was 0.18 mg/L.[10] The F levels in the serum in the present study for both the ICGN and ICR mice were higher than in the control group and higher than those in the renal insufficiency rats in our previous study.[7] Although this might affect the results, the indexes for evaluation were different between the studies.

The results of the study of the viabilities of the ICGN and ICR mice exposed to F showed all the ICGN mice exposed to F at 150 ppm died. Three of 7 ICGN mice exposed to F at 100 ppm also died. However, no ICR mice died. These results are in accordance with previous studies.[7,18] These results clearly demonstrate the enhanced toxic effects of F on the ICGN mice. In the ICGN mice exposed to 100 ppm in the previous study, increases in the thickness of the glomerular capillary walls and the mesangial matrixes in the kidneys were observed.[18] There were no pathological findings in the kidneys of the ICR mice or in the kidney function when evaluated by BUN and serum creatinine. However, it is still not clear whether or not mice with normal kidney function are affected by subacute exposure to F at 150 ppm in their drinking water. For example, BALB/c mice exposed to F at 125 ppm, with an increased serum F, showed a slight decrease in mRNA expression of TNFα (tumor necrosis factor-α) in splenic macrophages.

For femurs, there were no significant differences in any of the bone indexes among the ICGN groups. However, the mean bone mineral content and the bone

mineral density, demonstrated by ΣGS/D and $\Sigma GS/D^2$, in the left femur from male ICR mice exposed to F at 150 ppm were significantly higher than those from the control group. Also for the right femur from the male ICR mice exposed to 150 ppm F, the mean value of $\Sigma GS/D^2$ was significantly higher, and those for ΣGS/D and bone mineral content tended to be higher than in the control group. Male ICR mice exposed to F could be a better model of osteofluorosis. It should be noted that the observation period in this study of 1 mo was relatively short.

For the female ICR mice, there were no significant differences in the bone indexes between the control and the 150 ppm groups. However, the mineral content of the left femur from the female 150 ppm group tended to be higher than that of the control group. The mean value of ΣGS/D of the right femur from the 150 ppm group tended to be higher than that of the control group. This suggests that the effect of F on the bones of females was weaker than it was for males. In the previous NIH study,[9] increased osteoid was observed in the male mice exposed to F in the drinking water at 50 ppm for 6 mo. For females, an increased osteoid content was observed in the mice exposed to 100 or more ppm of F. The threshold dose for an increased osteoid content in the femur was 50 ppm for males and 100 ppm for females. The reasons for difference between the sexes were not clear, However, it is most likely to be due to female hormones. In an epidemiological study in Taiwan to determine the effects of chemical compounds including F on bone mineral density, being of the female sex was one of the significant negative variables for bone mineral density as revealed by multiple stepwise regression analyses.[20]

The reasons for there being no differences in the bone indexes in the ICGN mice were not clear. Some of the mice exposed to 100 or 150 ppm died before the end of the observation period. The mean body weight in the 100 ppm group was significantly lower than that of the control group. The deterioration of the general condition of the mice in the high-dose groups may affect the detection of alterations in the bones. In the study by Turner et al., even though the F in the blood was considered to be lower than that in the present study, an increase in osteoid bone was observed.[10] However, in their study, the exposure period and the indexes for evaluation were different.

In the present study, for teeth, a heterogeneity of calcification in the dentin was observed in the ICGN mice exposed to F at 150 ppm. In a previous NIH study,[9] the degeneration in the enamel of the incisors was observed in 5 of 5 mice exposed to F at 300 ppm in the drinking water. For females, the degeneration was observed in 1 of 3 mice exposed to F at 300 ppm and 2 of 2 mice exposed to F at 600 ppm. The results for teeth in the present study are in accordance with the results in the NIH study,[9] and the F concentration in the drinking water in the present study was lower than the doses used in the NIH study.[9] Although pathological changes in the enamel, such as heterogeneous structures of residual organic components, were also observed for the ICR mice, the ICGN mice may be a more adequate animal model of dental fluorosis.

It has been pointed out that work on rodents does not relate to humans because higher levels of fluoride are required to get bone and dental affects similar to those

in humans. In the present study with mice, 150 ppm of F in drinking water induced bone and dental effects. A previous study of rats, showed that they incorporate F in their bones far less efficiently than do humans because rats have four to five times less intestinal absorption of F than humans and a more than three times greater renal clearance of F.[21] The ability of rodents to excrete or metabolize F more efficiently than humans are able to explains the discrepancy in the F concentrations that induce osteofluorosis between humans and these experimental animals.

CONCLUSION

Thus, in conclusion, it is our contention that ICGN mice may be used as an adequate model of dental fluorosis. However, for the examination of F-induced osteofluorosis, ICR mice, particularly males, may be a better animal model. For examining the effects of F on osteofluorosis in ICR mice, exposure periods of 2 to 4 mo may be worthwhile. Further examination is warranted to definitively determine whether or not the alteration in the enamel in F-exposed ICGN mice is comparable to dental fluorosis.

ACKNOWLEDGEMENTS

We thank Dr Yuichi Ito, Department of Dentistry and Oral Surgery, Division of Medicine for Function and Morphology of Sensory Organs, Faculty of Medicine, Osaka Medical College, for advice on the pathological findings in the teeth. We also thank Robert E. Brandt, Founder, CEO and CME of MedEd Japan, for editing the manuscript.

REFERENCES

1 U.S. Public Health Service recommendation for fluoride concentration in drinking water for the prevention of dental caries. U.S. Department of Health and Human Services Federal Panel on Community Water Fluoridation. Public Health Reports 2015/11/30.
2 Tsunoda H. The environmental hazard by fluoride. In: Fujiwara M, Watanabe G, Takakuwa E, editors. Hygiene and public health. 2nd ed. Tokyo: Nankodou; 1985. pp. 478-81 [in Japanese].
3 Rawlani S, Rawlani S, Rawlani S. Assessment of skeletal and non-skeletal fluorosis in endemic fluoridated areas of Vidharbha region, India: a survey. Indian J Community Med 2010;35:298-301.
4 Wang C, Gao Y, Wang W, Zhao L, Zhang W, Han H, et al. A national cross-sectional study on effects of fluoride-safe water supply on the prevalence of fluorosis in China. BMJ Open 2012;2:e001564.
5 Samal AC, Bhattacharya P, Mallick A, Ali MM, Pyne J, Santra SC. A study to investigate fluoride contamination and fluoride exposure dose assessment in lateritic zones of west Bengal, India. Environ Sci Pollut Res 2015;22:6220-9.
6 Song HG, Gao JP, Wang CF, Chen CY, Yan XY, Guo M, et al. Sodium fluoride induces apoptosis in the kidney of rats thorough caspase-mediated pathways and DNA damage. J Physiol Biochem 2014;70:857-68.
7 Hosokawa M, Asakawa H, Kaido T, Sugaya C, Inoue Y, Tsunoda M, Itai K, Kodama Y, Sugita-Konishi Y, Aizawa Y. Deterioration of renal function in ICR-derived glomerulonephritis (ICGN) mice by subacute administration of fluoride in drinking water. Fluoride 2010;43:31-44.
8 Blaszczyk I, Birkner E, Gutowska I, Romuk E, Chlubek D. Influence of methionine and vitamin E on fluoride concentration in bones and teeth of rats exposed to sodium fluoride in drinking water. Biol Trace Elem Res 2012;146:335-9.

9 NIH NTP: National Institutes of Health. Toxicology and carcinogenesis studies of sodium fluoride (CAS no. 7681-49-4) in F344/N rats and B6C3F$_1$ mice. Research Triangle Park, NC. (NIH Publication No. 90-2848; National Toxicology Program Technical Report 393) 1990.
10 Turner CH, Owan I, Brizendine EJ, Zhang W, Wilson ME, Dunipace AJ. High fluoride intakes cause osteomalacia and diminished bone strength in rats with renal deficiency. Bone 1996;19:595-601.
11 Ormrod D, Miller T. Experimental uremia. Description of a model producing varying degrees of stable uremia. Nephron 1980;26:249-54.
12 Uchio K, Manabe N, Tamura K, Miyamoto M, Yamaguchi M, Ogura A, Yamamoto Y, Miyamoto H. Decreased matrix metalloproteinase activity in the kidneys of hereditary nephrotic mice (ICGN strain). Nephron 2000;86:145-51.
13 Zamberlan N, Radetti G, Paganini C, Gatti D, Rossini M, Braga V, Adami S. Evaluation of cortical thickness and bone density by roentgen microdensitometry in growing males and females. Eur J Pediatr 1996;155:377-82.
14 Matsumoto C, Kushida K, Sato Y, Yamazaki K, Denda M, Sumi Y, Inoue T, Yamashita K, Imose K. The second metacarpal bone density using the microdensitometry method. Nihon Seikeigeka Gakkai Zasshi 1992 (special issue);43:1141-7.
15 Majumdar KK, Sundarraj SN. Health impact of supplying safe drinking water on patients having various clinical manifestations of fluorosis in an endemic village of West Bengal. J Family Med Prim Care 2013;2:74-8.
16 Liu G, Ye Q, Chen W, Zhao Z, Li L, Lin P. Study of the relationship between the lifestyle of residents residing in fluorosis endemic areas and adult skeletal fluorosis. Environ Toxic Pharmacol 2015;40:326-32.
17 Zhang Y, Cheng R, Cheng G, Zhang X. Prevalence of dentine hypersensitivity in Chinese rural adults with dental fluorosis. J Oral Rehabil 2014;41:289-95.
18 Hosokawa M, Asakawa H, Kaido T, Sugaya C, Tsunoda M, Itai K, Kodama Y, Sugita-Konishi Y, Takata A, Yokoyama K, Aizawa Y. Fluoride in drinking water exacerbates glomerulonephritis and induces liver damage in ICR-derived glomerulonephritis mice. Toxicol Environ Chem 2011;93:2072-84.
19 Hosokawa M, Tsunoda M, Liu Y, Nakano K, Itai K, Tsunoda H, Yokoyama K, Aizawa Y. The immunotoxic effects of fluoride on mice after subacute administration by evaluating cytokine mRNA expression in splenocytes. Fluoride 2015;48:329-37.
20 Wu CH, Yang YC, Yao WJ, Lu FH, Wu JS, Chang CJ. Epidemiological evidence of increased bone mineral density in habitual tea drinkers. Arch Intern Med 2002;162:1001-6.
21 Whitford GM, Biles ED, Birdsong-Whitford NL. A comparative study of fluoride pharmacokinetics in five species. J Dent Res 1991;70:948-51.