DETERIORATION OF RENAL FUNCTION IN ICR-DERIVED GLOMERULONEPHRITIS (ICGN) MICE BY SUBACUTE ADMINISTRATION OF FLUORIDE IN DRINKING WATER

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SUMMARY: Sodium fluoride was administered at 0, 25, 50, 100, and 150 ppm F in drinking water for 4 weeks to Institute of Cancer Research (ICR) derived glomerulonephritis (ICGN) mice. Fluoride was also administered to ICR mice at 0 and 150 ppm. Blood was sampled from the tail artery of each mouse twice a week for the determination of blood urea nitrogen (BUN) and creatinine (CRE). All ICGN mice in the 150 ppm F group and 4 of 9 in the 100 ppm F group died before the end of four weeks, but no ICR control mice died. The mean values of BUN and CRE in the serum of the 150 ppm ICGN mice were significantly higher than those in the ICGN control mice at the end of the exposure period. The mean relative liver weight of the 150 ppm ICGN mice was significantly lower than that of the ICGN control mice. We conclude that F significantly exacerbates renal dysfunction.

Keywords: Blood urea nitrogen; Fluoride and glomerulonephritis; ICGN mice; Kidney dysfunction; Renal insufficiency; Serum creatinine.

INTRODUCTION

The physiological effects of fluoride (F) vary with genetic susceptibility.[1] Excessive F intake over time results in differing degrees of dental and osteo-fluorosis.[2] In China[2,3] and India,[4,5] many inhabitants who drink well water with high concentrations of F suffer from endemic skeletal fluorosis. In addition to bones and teeth, the kidney is a prime target organ of F toxicity.[6-9] Because F is filtered from the blood by the kidneys and excreted in the urine,[10] the quality of kidney function is very important and may be closely related to differences in susceptibility to F toxicity. Greater accumulation of F occurs in people who have impaired renal function and can affect them more seriously. It is also seems likely that F accumulation induces increased kidney damage.

In this research, we hypothesized that individuals with impaired kidney function have increased sensitivity to the toxic effects of F. For a laboratory study of this question, mice with a hereditary nephritic syndrome, derived from the Institute of Cancer Research (ICR) mice and known as ICR-derived glomerulonephritis (ICGN) mice, were chosen for this research to examine the effects of F on impaired kidney function by measuring blood urea nitrogen (BUN) and creatinine (CRE) in the blood.

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MATERIAL AND METHODS

Experimental animals: ICGN mice[11] that originated from a mutant of ICR mice were obtained from the National Institute of Health and Science, Tokyo, Japan. Mice that had BUN equal to or more than 36.0 mg/dL in the serum were used in the present study. ICR mice (Oriental Yeast Co., Ltd., Tokyo, Japan) were used as a normal kidney function control.

Fluoride exposure and BUN determination: The male ICGN mice at the ages of 11–14 wk were exposed to F ion at the concentration of 0, 25, 50, 100 (n = 9/group), and 150 ppm (n = 7/group) in the drinking water for 4 wk. The male ICR mice were aged 13 wk and were exposed to F at 0 and 150 ppm in the drinking water (n = 8 at 0 ppm and 6 at 150 ppm). For the stock solution a F ion concentration at 10,000 ppm was prepared by dissolving 22.1 g of NaF (MW = 41.99, Nacalai Tesque, Kyoto) in 1000 mL of low-F tap water (less than 0.8 mg/L).The solution was diluted with the low-F tap water to give F ion concentrations of 25, 50, 100, and 150 ppm. Mice were fed standard commercial rodent chow. Water intake and food consumption as well as body weight were monitored daily. Blood was sampled from the tail artery of each mouse twice a week for the determination of BUN in the serum. The blood was transferred to a 1.5 mL polypropylene tube and centrifuged at 6000 rpm for 3 min. The BUN concentration in the resulting serum were determined with a urea nitrogen kit, Fuji Dry Chem Slide, BUN-P Ⅲ (Fuji Film Medical, Tokyo, Japan) using the Fuji 5500V (Fuji Dry Chem, Fuji Film Medical). After the observation period, the mice were euthanized with ether. The internal organs: heart, lung, liver, kidney, and spleen were removed and weighed. When mice died during the observation period, the body weight was recorded, and the internal organs were also removed and weighed.

CRE determination: The male ICGN mice (13 or 14 wk) were administered F in the drinking water at concentrations of 0, 25, 50, 100, and 150 ppm F ion (3/group) in the drinking water for 4 wk. The male ICR mice (13 or 14 wk) were also exposed to F ion at 0 and 150 ppm (n = 5 at 0 ppm and 3 at 150 ppm) in the drinking water for 4 wk. The blood was sampled from the tail artery twice a week for one month. The CRE in the serum was determined by blood CRE kit, Fuji Dry Chem Auto Slide, CREIII (Fuji Film Medical) by using Fuji Dry Chem 5500V.

Fluoride in the serum: The female ICGN mice (18 wk) were administered F at 0, 25, 50, 100, and 150 ppm in the drinking water for 4 wk (n = 9 at 0 ppm, 6 at 25 ppm, 14 at 50 ppm, 9 at 100 ppm, and 10 at 150 ppm). The female ICR mice (18 wk) were exposed to F at 0 and 150 ppm in the drinking water (n = 4 at 0 and 150 ppm). The mice were killed by decapitation and the blood was sampled. F concentrations in the serum were determined for the ICGN and ICR mice by a flow-injection apparatus with a F ion-selective electrode as a detector.[12,13]

Statistical analyses: The deaths of mice during treatment were recorded, and the viability was calculated daily. The mean values of the daily food and water intake for each mouse were calculated as well as the mean values of daily food and water per body weight. Then, the mean values of daily food and water intake and those

per body weight in each group were calculated. At the end of the treatment period the mean values of body weight, relative organ weight, BUN, CRE, and F in the serum were also calculated. When a mouse died, the data on the day of death or nearest to the day of death was assigned. These mean values were compared by one-way ANOVA (analysis of variance) using Statview 5.02 v (SAS, Cary, CA), followed by the Student-Newman-Keuls test used as a post hoc test (significance level, $p < 0.05$).

RESULTS

The mean values of the daily intake of food and water and the mean values of the intake of food and water per body weight among ICGN mice and ICR mice are shown in Table 1.

Table 1. Mean values of the daily intake of food and water and the mean values of the intake of food and water per body weight among ICGN mice and ICR mice exposed to F in drinking water (mean ± standard error)

Mice	F Concentration	Food	Water
		Daily intake of food and water (g)	
ICGN	0 ppm	4.64 ± 0.19	7.70 ± 0.19
	25 ppm	4.73 ± 0.28	8.21 ± 0.40
	50 ppm	4.79 ± 0.32	7.69 ± 0.28
	100 ppm	4.35 ± 0.36	7.62 ± 0.48
	150 ppm	2.69 ± 0.58 *†‡§	5.10 ± 0.99 *†‡§
ICR	0 ppm	5.10 ± 0.14	7.02 ± 0.38
	150 ppm	5.14 ± 0.15	6.66 ± 0.44
		Intake of food and water per body weight (g/g bw)	
ICGN	0 ppm	0.156 ± 0.012	0.256 ± 0.012
	25 ppm	0.165 ± 0.012	0.283 ± 0.017
	50 ppm	0.159 ± 0.013	0.267 ± 0.012
	100 ppm	0.156 ± 0.011	0.273 ± 0.022
	150 ppm	0.116 ± 0.024	0.224 ± 0.041
ICR	0 ppm	0.124 ± 0.001	0.171 ± 0.008
	150 ppm	0.125 ± 0.002	0.161 ± 0.011

$p = 0.0013$ for food and $p = 0.0013$ for water by ANOVA for ICGN mice. $^{*}p < 0.05$ compared to 0 ppm, $^{\dagger}p < 0.05$ compared to 25 ppm, $^{\ddagger}p < 0.05$ compared to 50 ppm, $^{\S}p < 0.05$ compared to 100 ppm by the Student-Newman-Keuls test.

The mean value of the intake of food and water in the 150 ppm group was significantly lower than those in the other groups. The mean value of the intake of food and water per body weight was not significantly different among the groups for either ICGN or ICR mice. Based on the water intake, the mean value of F intake per body weight for the 4 wk period was calculated for ICGN mice as follows: 7.08 mg/kg in the 25 ppm group, 13.4 mg/kg in the 50 ppm group, 27.3 mg/kg in the 100 ppm group, and 33.6 mg/kg in the 150 ppm group. The mean F intake per body weight for the ICR mice exposed to 150 ppm F was 24.2 mg/kg.

For the ICGN mice exposed to F, the viability for each group in the experiment for BUN determination over the observation period is shown in Figure 1. All seven ICGN mice exposed to 150 ppm F died during the observation period. Four of nine ICGN mice exposed to 100 ppm F died over the observation period. All ICGN mice exposed to 150 ppm F in other experiments for CRE and F concentration in serum also died during the observation period, whereas no ICR control mice exposed to 150 ppm F died (data not shown).

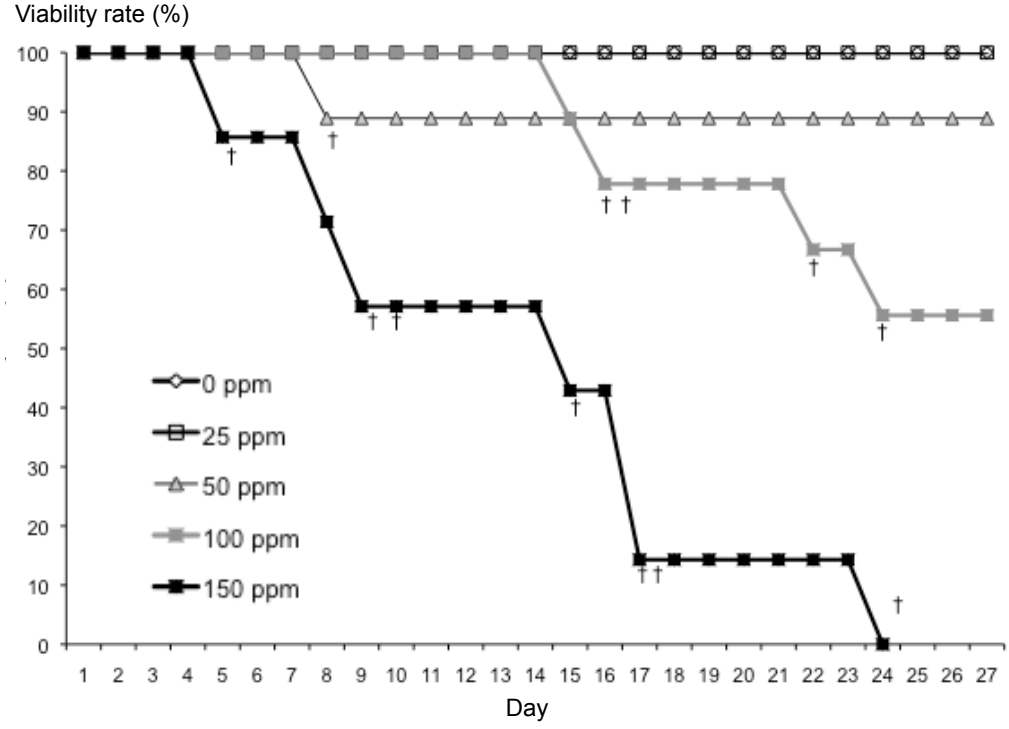

Figure 1. Viabilities of ICGN mice and ICR mice exposed to 0, 25, 50, 100, and 150 ppm F in their drinking water for 1 month. † Death.

The mean values of the body weights at the end of the observation period for the ICGN and ICR mice are shown in Figure 2. The mean body weight of the 150 ppm ICGN group was significantly lower than those in all the other groups. The mean body weight of mice at their deaths during the study period was 16.9 g. There was no significant difference in body weight between the ICR mice exposed to 0 and 150 ppm F.

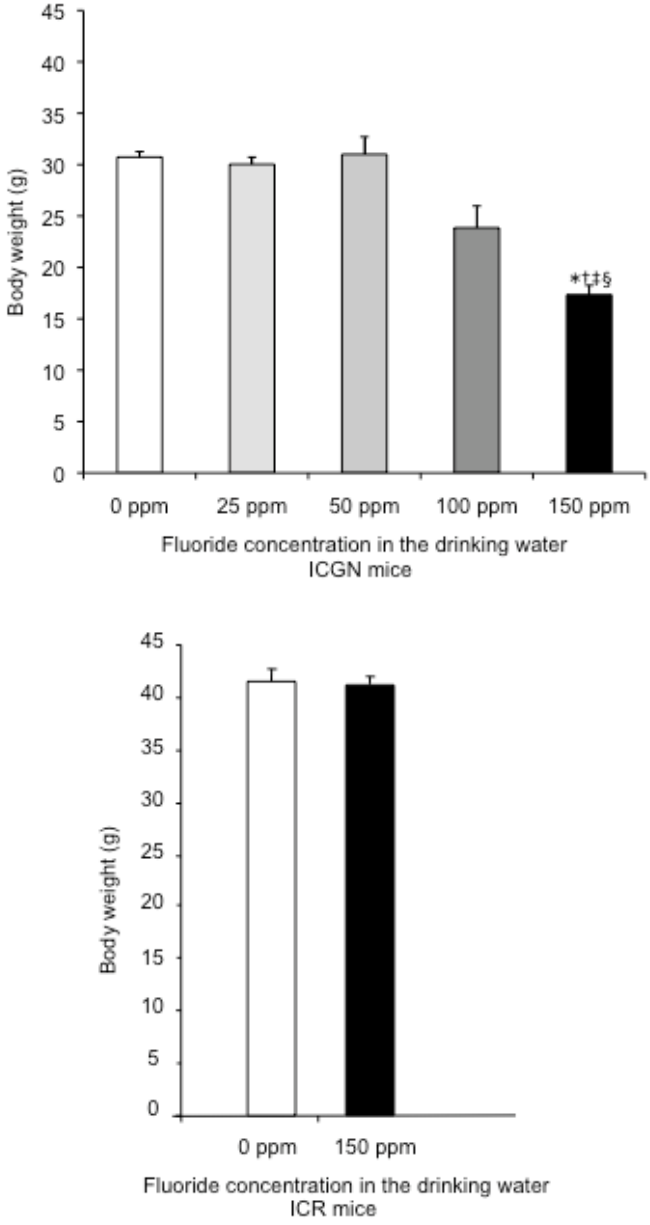

Figure 2. Mean values of the weight of ICGN mice and ICR mice exposed to F at the end of the observation period. When a mouse died, the data on the day nearest to the day of death were assigned as they were for all of the mice in the present study. Each bar represents the mean value, and error bars represent standard errors. $p = 0.0001$ by ANOVA for ICGN mice. $^{*}p < 0.05$ compared to 0 ppm, $^{\dagger}p < 0.05$ compared to 25 ppm, $^{\ddagger}p < 0.05$ compared to 50 ppm, $^{\S}p < 0.05$ compared to 100 ppm by the Student-Newman-Keuls test.

The mean values of the BUN at the end of the observation period are shown in Figure 3. For the dead ICGN mice exposed to 100 or 150 ppm F, the values nearest to the day of death were used as they were for all of the mice in the present study.

For the ICGN mice, the mean value of BUN in the 150 ppm group was significantly higher than those in all other groups. There was no significant difference in BUN between the ICR mice exposed to 0 and 150 ppm.

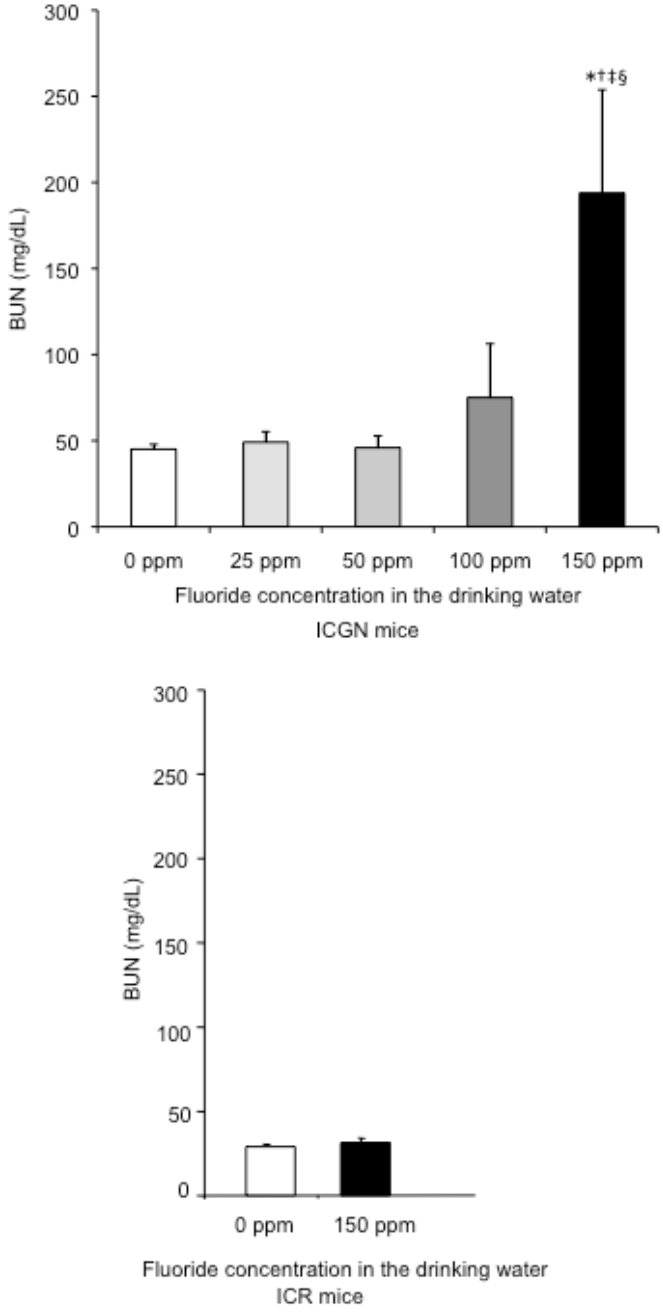

Figure 3. Mean values of BUN of ICGN mice and ICR mice exposed to F at the end of the observation period. When a mouse died the data on the day nearest to the day of death were assigned. Each bar represents the mean value, and error bars represent standard errors. p = 0.0003 by ANOVA for ICGN mice. *$p < 0.05$ compared to 0 ppm; †$p < 0.05$ compared to 25 ppm; ‡$p < 0.05$: compared to 50 ppm; §$p < 0.05$, compared to 100 ppm by the Student-Newman-Keuls test.

Each serum BUN value for each ICGN mouse at the sampling points is shown in Figure 4 for the 100 ppm and the 150 ppm groups. The BUN value increased rapidly in most mice in the 150 ppm group before death. Two of 4 dead ICGN mice exposed to 100 ppm F also showed rapid increase in the BUN value before death.

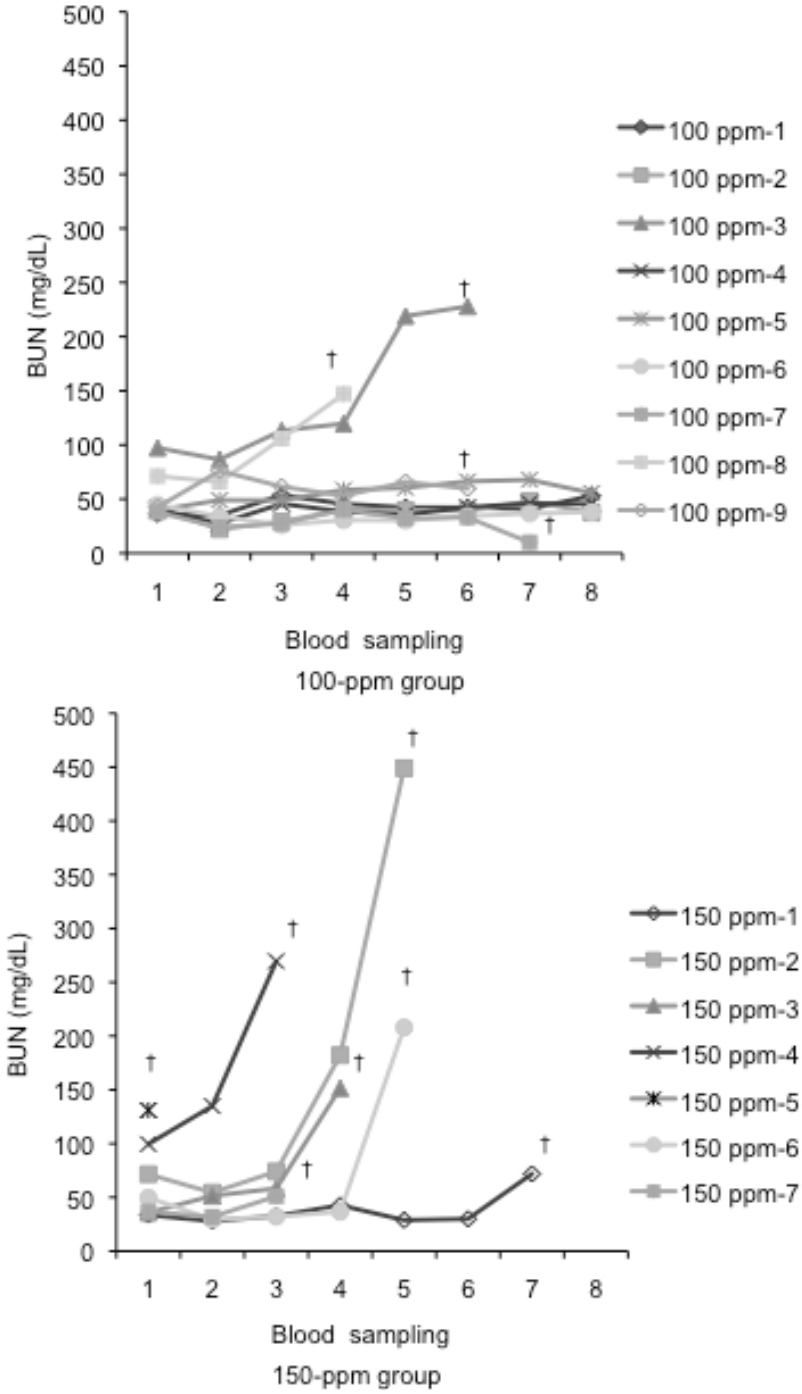

Figure 4. BUN in the serum of ICGN mice exposed to 100 ppm or 150 ppm F in drinking water at sampling points.Blood sampling 1, day 1; 2:, day 4; 3, day 8; 4, day 11; 5, day 15; 6, day 18; 7, day 22; 8, day 25. † Death.

The liver weight and relative liver weight of ICGN mice are shown in Figure 5. Both the mean liver weight and relative liver weight in the 150 ppm group were significantly lower compared with those in all other groups. There were no significant differences in liver weight and relative liver weight between the ICR mice exposed to 0 and 150 ppm F (data not shown).

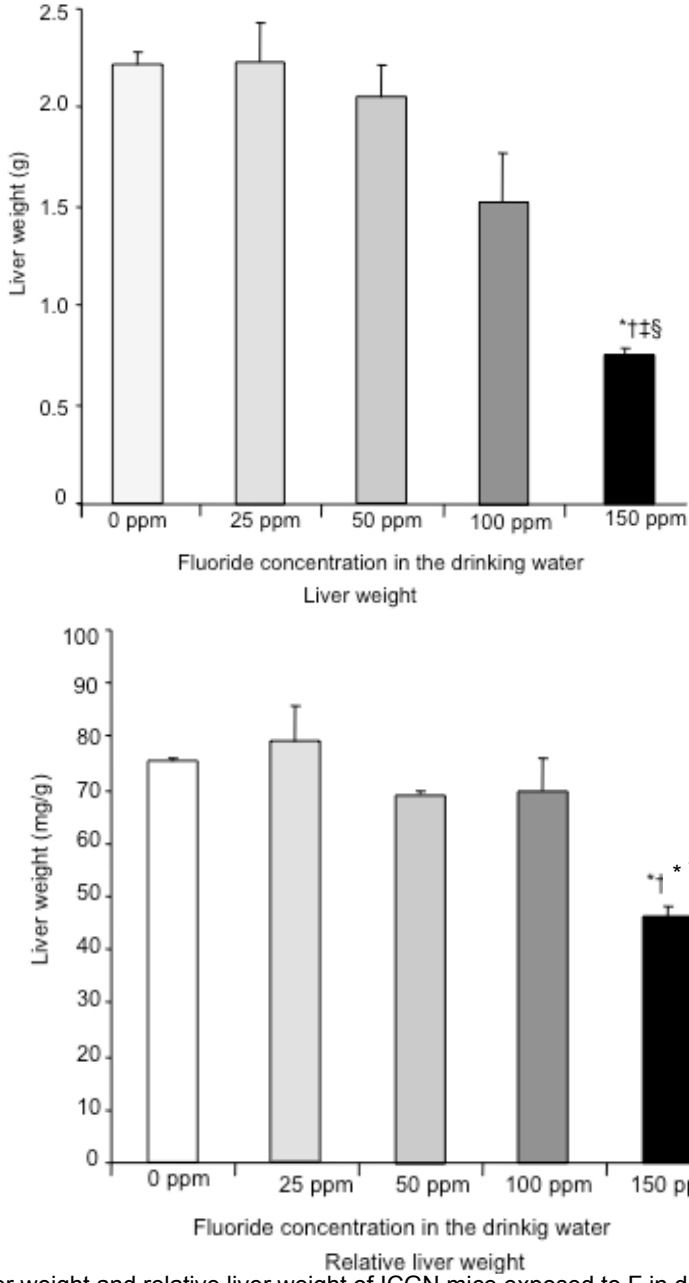

Figure 5. Liver weight and relative liver weight of ICGN mice exposed to F in drinking water. When a mouse died, the data on the day nearest to the day of death were assigned. Each bar represents the mean value, and error bars represent standard errors. p = 0.0001 for liver weight and p = 0.0009 for relative liver weight by ANOVA. *$p < 0.05$ compared to 0 ppm, †$p < 0.05$ compared to 25 ppm, ‡$p < 0.05$ compared to 50 ppm, §$p < 0.05$, compared to 100 ppm by the Student-Newman-Keuls test.

For other organs, the organ weight and relative organ weight of ICGN and ICR mice are shown in Table 2.

Table 2. The organ weights and relative organ weights (mean ± standard error) of ICGN or ICR mice exposed to F for 4 weeks

Mice	F concentration	Kidney	Lung	Heart	Spleen
		Organ weights (g)			
ICGN	0 ppm	0.512 ± 0.082	0.205 ± 0.015	0.172 ± 0.015	0.196 ± 0.023
	25 ppm	0.540 ± 0.120	0.310 ± 0.127	0.308 ± 0.157	0.169 ± 0.010
	50 ppm	0.506 ± 0.033	0.200 ± 0.014	0.161 ± 0.009	0.144 ± 0.019
	100 ppm	0.424 ± 0.148	0.273 ± 0.131	0.265 ± 0.123	0.081 ± 0.045 *
	150 ppm	0.321 ± 0.027	0.171 ± 0.041	0.102 ± 0.018	0.032 ± 0.008 *†‡
ICR	0 ppm	0.526 ± 0.069	0.257 ± 0.063	0.240 ± 0.078	0.220 ± 0.038
	150 ppm	0.483 ± 0.079	0.243 ± 0.071	0.220 ± 0.067	0.195 ± 0.074
		Relative organ weights (mg/g bw)			
ICGN	0 ppm	17.2 ± 2.8	6.9 ± 0.5	5.7 ± 0.4	6.6 ± 0.8
	25 ppm	19.0 ± 4.1	10.9 ± 4.4	10.9 ± 5.5	6.0 ± 0.3
	50 ppm	17.2 ± 0.7	6.9 ± 0.7	5.5 ± 0.3	5.1 ± 0.8
	100 ppm	17.8 ± 4.3	11.1 ± 4.2	11.0 ± 3.8	3.5 ± 1.5
	150 ppm	19.2 ± 1.3	10.2 ± 2.4	6.2 ± 1.2	2.0 ± 0.5 *†
ICR	0 ppm	13.1 ± 1.8	6.5 ± 1.7	6.1 ± 2.1	5.6 ± 1.0
	150 ppm	11.6 ± 1.7	5.8 ± 1.6	5.3 ± 1.5	4.6 ± 1.7

$p = 0.001$ for spleen weight and $p = 0.011$ for relative spleen weight for ICGN mice by ANOVA.

*$p < 0.05$ compared to 0 ppm, †$p < 0.05$ compared to 25 ppm, ‡$p < 0.05$ compared to 50 ppm.

The mean spleen weight of ICGN mice in the 150 ppm group was significantly lower compared to those in the 0, 25, and 50 ppm groups. The mean spleen weight in the 100 ppm group was significantly lower compared to that in the 0 ppm group. The mean relative spleen of the ICGN mice in the 150 ppm group was significantly lower compared with that in the 0 and 25 ppm groups.

The mean values of CRE at the end of the observation period are shown in Figure 6.

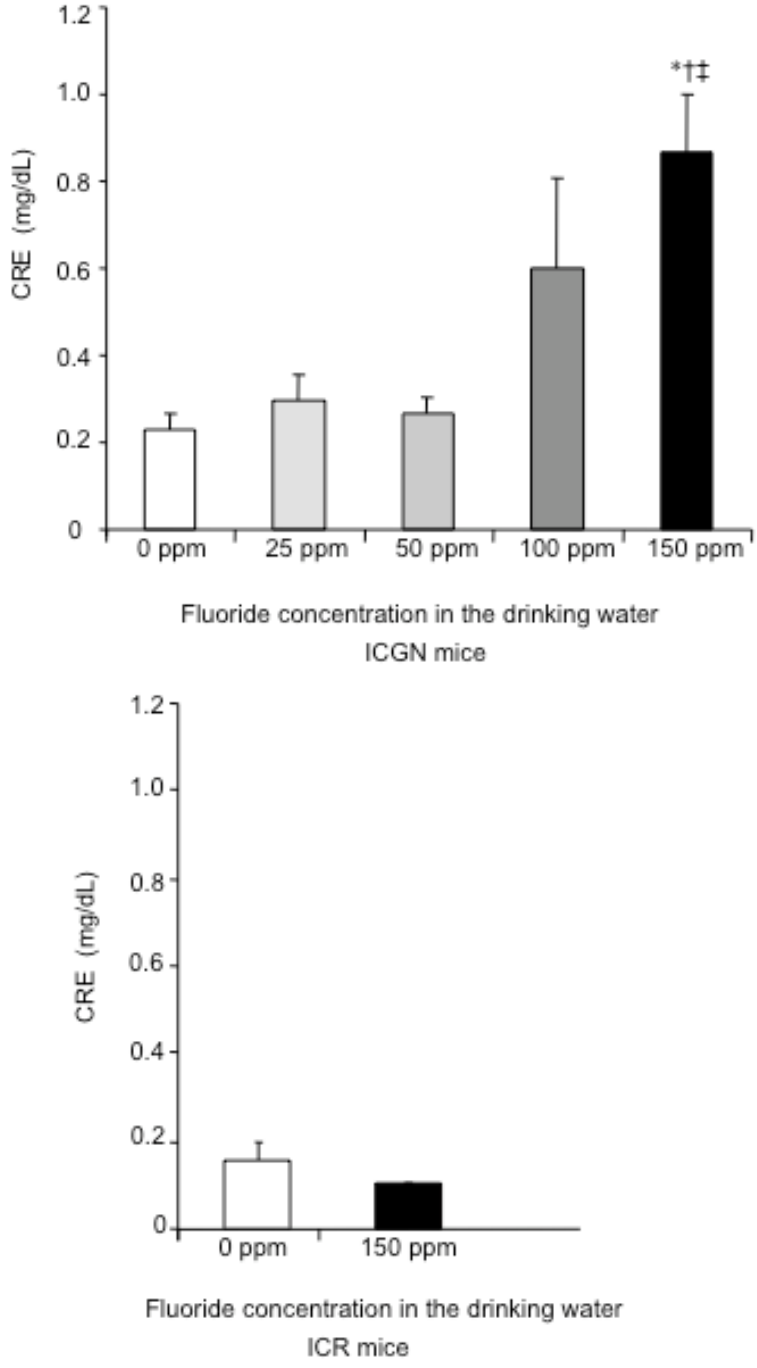

Figure 6. Mean values of CRE in the serum of ICGN and ICR mice at the end of the observation period. When a mouse died, the data on the day nearest to the day of death were assigned. Each bar represents the mean value, and error bars represent standard errors. $p = 0.0124$ by ANOVA for ICGN mice. *$p < 0.05$ compared to 0 ppm, $^{\dagger}p < 0.05$ compared to 25 ppm, $^{\ddagger}p < 0.05$ compared to 50 ppm by the Student-Newman-Keuls test.

The mean value of CRE in the 150 ppm ICGoup was significantly higher than those in all other groups. There was no significant difference in CRE between the ICR mice exposed to 0 and 150 ppm.

Figure 7 shows the serum F concentration in female mice at the end of the treatment period.

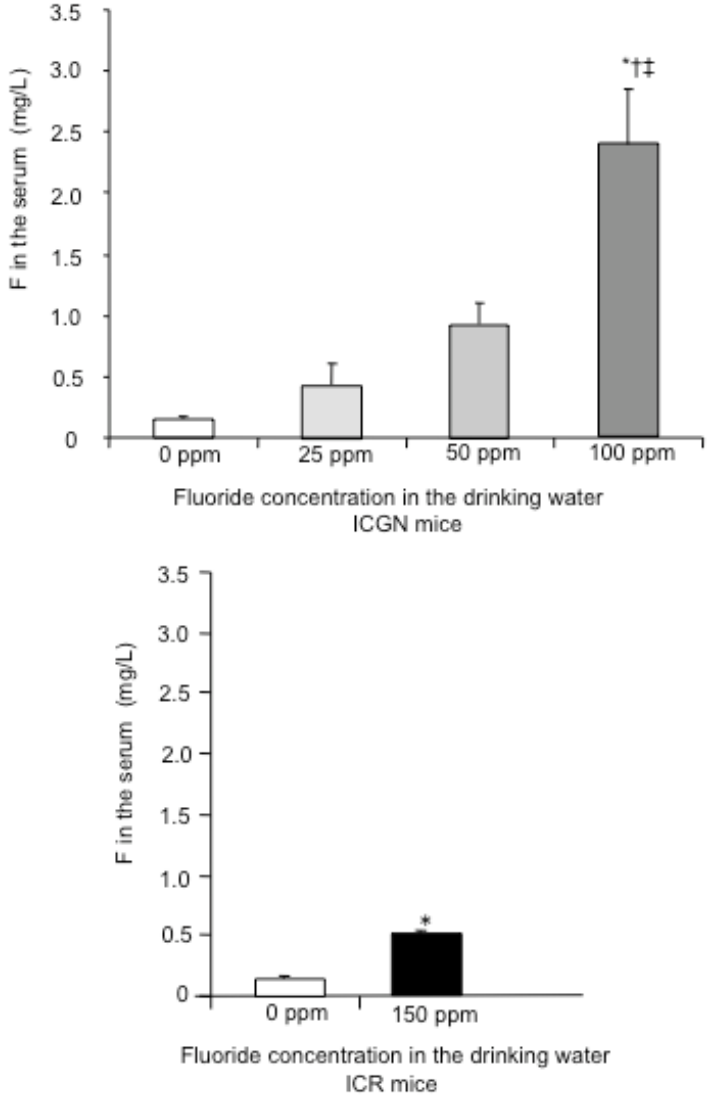

Figure 7. F concentration in the serum of female CGN and ICR mice exposed to F in their drinking water. All ICGN mice exposed to 150 ppm F died before the end of the observation period, so the F concentration in the serum could not be determined. Each bar represents the mean value, and error bars represent standard errors. p = 0.0124 by ANOVA for ICGN mice. *p < 0.05 compared to 0 ppm, †p < 0.05 compared to 25 ppm, ‡p < 0.05 compared to 50 ppm by the Student-Newman-Keuls test. *p < 0.05 compared to 0 ppm for ICR mice by t test.

Because all ICGN mice exposed to 150 ppm had died before the end of the period, the F concentrations in the serum could not be determined. The mean F

concentration in the serum in the ICGN mice exposed to 100 ppm was significantly higher than those in other ICGN groups. As expected, the mean serum F level in the ICR mice exposed to 150 ppm F was significantly higher than that in the 0-ppm F group. The actual serum level of F in the ICR mice exposed to 150 ppm was 0.50 mg/L, which was close to the serum F concentration in the ICGN mice exposed to 25 ppm F (0.43 mg/L).

DISCUSSION

Many laboratory studies on the effects of F on kidney function have been reported. For example, newborn Sprague-Dawley rats receiving a single i.p. injection of 0, 30, or 48 mg NaF/kg bw on postnatal day 1, 8, 15, or 29 showed alterations in renal function and histological changes 24, 48, and 120 hr after treatment.[14] Among the rats that received 48 mg/kg NaF (21.7 mg F ion) on day 29, a decrease in the urine osmotic pressure, glycosuria, hematuria, and histological changes in the proximal tubules were observed. Stawiarska-Pięta et al.[15] administered 3 mg F/kg bw/day to rabbits in their drinking water with a diet supplemented with 2.0 g of cholesterol/kg for 3 months and observed foci of renal tubule cell steatosis. Zhan et al.[16] administrated a basal diet supplemented with F at 0, 100, or 200 mg/kg diet to pigs for 50 days. The mean value of BUN in the 100-mg and 200-mg groups was significantly higher than that in the control, as was the mean CRE in the serum in the 200 mg group. In a 30 day study on mice, Chinoy[17] administered NaF at 5 mg/kg bw[17] and found that the level of CRE in the kidney decreased. Thus, renal damage is one of major adverse effects of F.

For effects of F on other organs, Bouaziz et al.[18] examined liver damage induced by F administration. Pregnant Wistar mice were exposed to 500 ppm NaF (226 ppm F) in the drinking water from the 15th day of pregnancy until day 14 after delivery. Alanine transaminase (ALT) and aspartate transaminase (AST) in the serum increased significantly for both the dams and the first-generation offspring (F1) mice. Histological changes including intensive ballooning and infiltration of mononuclear cells in the liver were observed among both the dams and the F1 mice. Kour et al.[19] reported pathological changes in the liver of guinea pigs exposed to NaF at concentrations of 0, 500, and 1000 ppm in the drinking water for 1, 2, or 3 months. Those exposed to 1000 ppm NaF for 1 month had focal necrosis of the liver, and those exposed for 2 months had fatty changes in liver cells and reticuloendothelial cell hyperplasia. We therefore also checked the liver weight in our investigation.

The ICGN mouse[11] originated from the ICR mouse and is considered a good model of human idiopathic renal insufficiency. In the present study, we set the F concentrations in the drinking water of the mice at 25 ppm and more, since it is possible for the people to be exposed to the equivalent of about 25 ppm F in drinking water in F-endemic areas in China and India. The 150 ppm F group of the ICGN mice showed a rapid decrease in body weight, and all these mice died during the observation period. In contrast, among the 150 ppm F group of ICR mice, there was no significant decrease in body weight compared to their 0 ppm F

control, and none of these mice died. At the end of the study, the mean values of BUN and CRE in the 150-ppm F exposed ICGN mice were significantly higher than those in all the other ICGN groups. These results are in agreement with those of Zhan et al. for young pigs.[16] The values of BUN and CRE in the serum increased shortly before death in the many ICGN mice that died, suggesting that a severe decrease in body weight and death were closely related to the deterioration of kidney function. This severe renal damage with rapid increase in the BUN and CRE in the serum by oral administration of F to the ICGN mice occurred at a dose similar to that caused by i.p. administration to rats by Daston et al.[14]

The significant increase in the serum F concentration in the female ICGN mice exposed to 100 ppm F indicated a decrease in F excretion by the kidney. As noted in the Results, the F concentration in the serum of the ICR mice exposed to 150 ppm F was similar to that of the ICGN mice exposed to 25 ppm F. Thus the decrease in excretion of F by the ICGN mice was associated with a deterioration of the general condition and function of the kidneys. People with renal insufficiency should therefore take care to restrict their F intake.

Compared to the liver damage from F reported by Bouaziz et al.,[18] the doses of F in the present study were relatively low. However, the ICGN mice exposed to 150 ppm F had significantly lower liver weight and relative liver weight compared to the ICGN control. In contrast, no significant changes in liver weight or relative liver weight in the ICR mice exposed to 150 ppm F were observed. It therefore appears that liver toxicity was induced by relatively lower doses of F for the ICGN mice with impaired kidney function. Thus people with impaired kidney function may also experience liver toxicity from F. Hence determination of ALT and AST in the serum in the ICGN mice exposed to F is of interest and warrants further study. In addition, it is desirable to examine the liver of the F-exposed ICGN mice for pathological changes.

As noted in the Results, the inhibitory effects of F were observed on the spleen weight among internal organs. In a previous *in vitro* study by Hosokawa et al.,[20] cell death of macrophages was observed at 1 mM F. In the present study, significant decrease in the relative spleen weight was observed only in the 150-ppm group of the ICGN mice, perhaps because the highest mean serum concentration of F in the 100 ppm F group of the ICGN mice was 0.13 mM (2.47 mg F/L). For neurotoxicity, alterations in neurotransmitters were reported in BALB/c mice exposed to F.[21] The neurotoxicity may become strong in the ICGN mice exposed to F. It is, therefore, of interest to determine whether this might be the case or not.

In the present study, the amounts of blood collected from the mice were not sufficient for simultaneous determination of BUN, CRE, and F concentrations in the serum. In addition, the F concentrations in the serum were the data for female mice because of the limitation for getting a sufficient number of ICGN mice. It would be useful to check the alterations in serum F concentrations over the observation period and the changes in correlation between F and BUN or CRE. In addition, there were no data for F concentration in the rodent chow. It is useful to

determine F concentration in the rodent chow to evaluate the intake of F from food and water.

In conclusion, all the kidney impaired ICGN mice exposed to 150 ppm F died in less than a month, and the kidney function in this group deteriorated significantly, since the mean values of BUN and CRE in the serum were dramatically increased. People with renal insufficiently should therefore be careful to avoid excessive exposure to F.

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