

THE EFFECTS ON RENAL FUNCTION, IN INSTITUTE OF CANCER RESEARCH-DERIVED GLOMERULONEPHRITIS (ICGN) MICE, OF THE SUBACUTE ADMINISTRATION OF THE FLUORIDE ION IN DRINKING WATER

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ABSTRACT: The fluoride ion (F) is a known environmental pollutant which induces renal damage in experimental animals. Since F is filtered from the blood by the kidneys and excreted in the urine, its toxicity is expected to be enhanced when renal function is impaired. The objective of this study was to obtain basic information on changes in the renal function of ICR-derived glomerulonephritis (ICGN) mice exposed to F via drinking water by evaluating the blood urea nitrogen (BUN), the urinary protein and creatinine, and the creatinine clearance. F was administered for four weeks in drinking water to ICGN mice at 0, 50, 100, and 150 ppm and to ICR mice at 0, 100, and 150 ppm. Blood was sampled from the tail artery and the urine was sampled by a metabolic cage for each mouse three days after the start of the F exposure (first sampling) and at then at weekly intervals until the end of the experimental period. When a mouse died prior to the end of the 4 week period, the data from the weekly samples taken closest to the day of the death were used. For the ICGN mice, at the end of the experimental period, the mean BUN in the 150 ppm group was significantly higher ($p<0.05$) than the levels in the 0 and 50 ppm groups. For the ICR mice, after 3 days, the BUN in the 150 ppm group was significantly higher ($p<0.05$) than the values in the 0 and 100 ppm groups. These results clearly demonstrate the serious toxic effects of ≥ 100 ppm F in the drinking water for mice with impaired kidney function. The early increase in the BUN of the ICGN mice provides an adequate index of the F-induced deterioration in kidney function.

Keywords: Blood urea nitrogen; Creatinine clearance; ICR-derived glomerulonephritis mice; Urinary F concentrations.

INTRODUCTION

The fluoride ion (F) is an environmental pollutant that has contaminated ground water in the People's Republic of China,¹ India,² Iran,³ and Argentina.⁴ Many people who drink well water with a high F concentration suffer from endemic fluorosis, and many epidemiological studies have shown that the long-term consumption of this water causes dental and skeletal fluorosis.^{3,5} In addition to the toxic effects of F on teeth and bones, 100 ppm of F in the drinking water of experimental animals induces renal damage.^{6,7} Moreover, since F is filtered from

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the blood by the kidneys and excreted in the urine, it is expected that the toxicity of F will be enhanced when the renal function is impaired.

In our previous animal study,⁸ a greater accumulation of F occurred in the Institute of Cancer Research (ICR)-derived glomerulonephritis mice (ICGN), which have impaired renal function,⁹⁻¹⁰ than in the control ICR mice with normal kidney function. F affects ICGN mice more severely than control mice^{8,11} and all the ICGN mice exposed to 150 ppm F in their drinking water died within four weeks. The mean blood urea nitrogen (BUN) and serum creatinine (CRE) values in the ICGN mice exposed to 150 ppm F in their drinking water were significantly higher than those in control mice.⁸

These studies did not include any information on the urine of the mice, such as the urinary levels of protein and creatinine. In addition, there was no information on the effect in animals with impaired renal function of F on the creatinine clearance, an index for glomerular filtration. In order to obtain information which might be useful in understanding the effects of F in glomerulonephritis patients in F-contaminated areas, we further investigated the effects of F on renal function in ICGN mice with impaired renal function.

The objective of the present study was to obtain basic information on renal function in ICGN mice exposed to F via drinking water by evaluating the blood urea nitrogen (BUN), the serum creatinine (CRE), the level of urinary protein, and the creatinine clearance.

MATERIALS AND METHODS

The experimental mice and the F exposure: The mice with impaired renal function were male 11–12-weeks-old ICGN mice obtained from the National Institute of Health and Science (Tokyo, Japan). Male ICR mice with normal renal function were used as controls (CLEA Japan, Inc., Tokyo). The initial serum BUN levels in the ICGN and the ICR mice were ≥ 36.0 mg/dL and ≤ 36.0 mg/dL, respectively. The mice (5 per group) were exposed for four weeks to F in the drinking water. The ICGN mice were exposed to F at concentrations of 0, 50, 100, or 150 ppm and the ICR mice were exposed to F at concentrations of 0, 100, or 150 ppm. Both groups had *ad libitum* access to water and the daily water intake was checked twice a week. The care and treatment of the mice were in accordance with the guidelines established by the Animal Experimentation and Ethics Committee of the Kitasato University School of Medicine and were approved by the Committee.

Sampling the body weight and the urine: The body weight of each mouse was checked twice a week. The urine of each mouse was sampled with a metabolic cage once a week. Each mouse was housed in a metabolic cage for 24 hr only once a week because mice have been reported to be stressed while in these cages.¹² To avoid pieces of rodent chow and feces falling into the urine, a stainless steel filter was put into the collecting tube at the bottom of the metabolism cage.

Determining the protein and creatinine levels in the urine: The protein level in the urine was determined by the Quick Start Bradford Assay Kit (Bio-RAD,

Tokyo, Japan). The standard protein or sample solutions (triplicate for each sample) were added to 200 μ L of a 5-times diluted Bradford reagent in a 96-well microplate. The microplate was incubated for 5 min at room temperature. The absorbance at 595 nm was measured using a Power Scan TH (BioTAK, Tokyo) and the protein concentrations were determined by a standard curve. The creatinine concentration in the urine was determined by the enzyme method¹³⁻¹⁵ (Special Reference Laboratories, Tokyo).

Determination of the serum BUN and CRE: Blood was sampled from the tail artery once a week and transferred to a 1.5 mL polypropylene tube and centrifuged at 6000 rpm for 5 min. The BUN and CRE concentrations in the serum were determined with a urea nitrogen kit, Fuji Dry Chem Slide, BUN-P III and Fuji Dry Chem Slide, and CRE-P III (Fuji Film Medical, Tokyo Japan) using the Fuji 5500V (Fuji Dry Chem, Fuji Film Medical).

Creatinine clearance: Creatinine clearance was calculated with the following formula:

$$\text{Creatinine clearance} = \frac{\text{Urinary creatinine concentration (mg/mL)} \times 24 \text{ hr urine volume (mL)}}{\text{Serum creatinine concentration (mg/mL)} \times 24 \times 60}$$

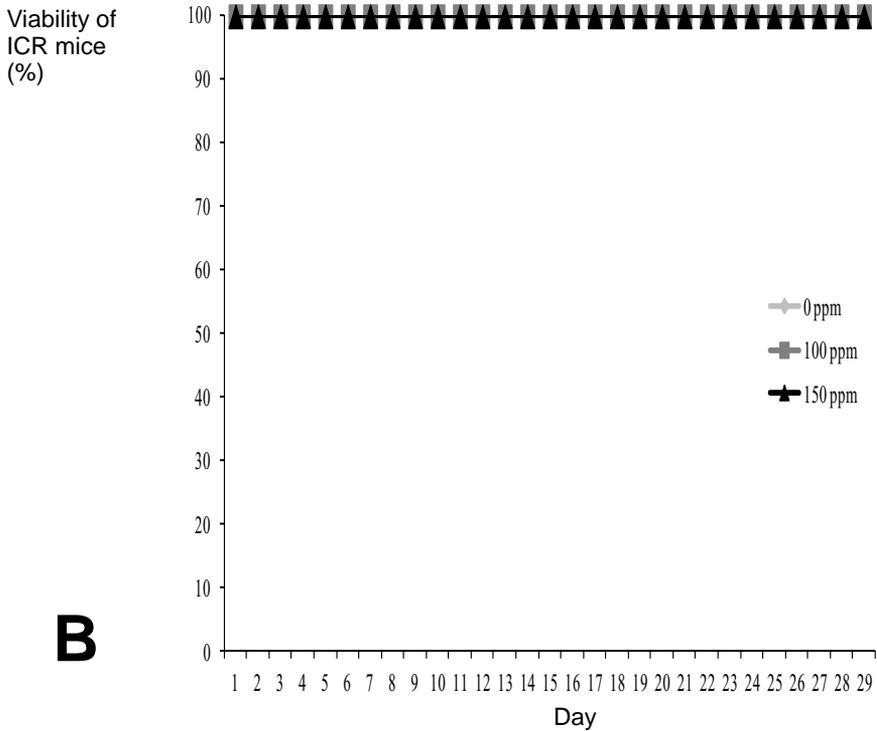
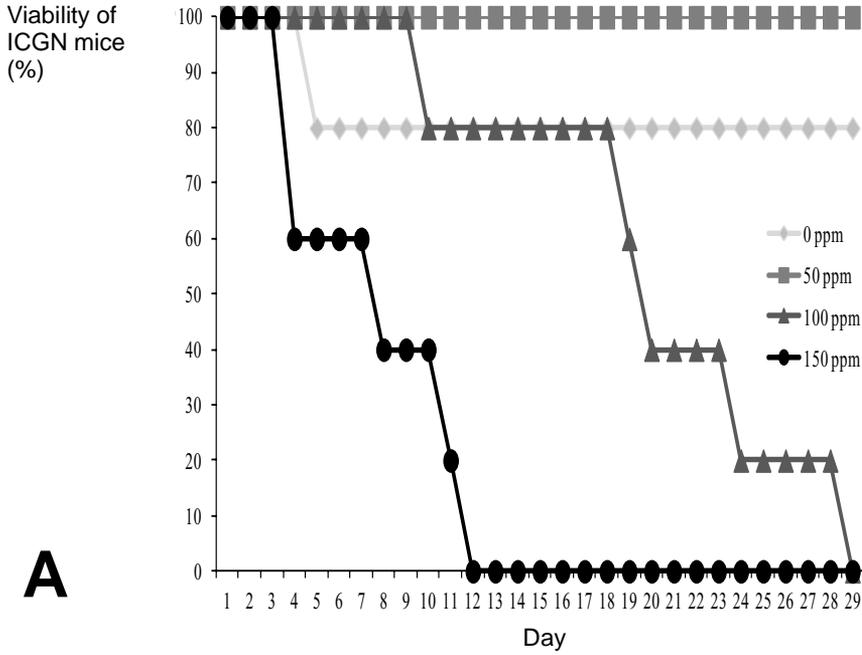
(mL/min)

Analysis of the F concentration in the urine: The concentration of F in the diluted serum was determined by a flow injection apparatus with an F-selective electrode as a detector¹⁶⁻¹⁸ (Fluoride Analysis Unit 2000, Daiwa Denshi, Kyoto, Japan). Each urine sample was diluted with distilled water according to its F concentration. Each sample was measured twice and the mean was calculated for each sample.

Statistical analyses: The mean value of the final body weight of each group was calculated. The ICGN and ICR groups were compared by a one-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls test. ANOVA was used to compare the values in the two groups, after three days of exposure (first sampling) and again after 4 weeks on the last sampling day, of the urinary protein, urinary CRE, creatinine clearance, urinary F, serum BUN, serum CRE, and the creatinine clearance. Since the F concentrations in the urine from the ICGN mice varied widely, they were analyzed by the non-parametric Kruskal-Wallis test. Statview J-5.0 software (SAS Institute, Cary, NC, USA) was used for all statistical analyses and results were considered significant at $p < 0.05$.

RESULTS

Figures 1A and 1B show the viability of the ICGN and ICR mice exposed to various concentrations of F (0, 50, 100, and 150 ppm) in their drinking water. All the ICGN mice exposed to 100 and 150 ppm F died during the observation period, while no ICR F-exposed mice died.



Figures 1A and 1B. The viability of the (A) ICGN and (B) ICR mice exposed to various concentrations of the fluoride ion (0, 50, 100, and 150 ppm) in their drinking water.

Table 1 shows the body weights of ICGN mice and ICR mice exposed to F at the end of observation period. When a mouse died prior to the end of the 4 week period, the data from the weekly samples taken closest to the day of the death were used. The mean body weights of ICGN mice exposed to 100 and 150 ppm F were significantly lower than those of the ICGN mice exposed to 0 and 50 ppm F. The mean body weights of the ICR mice exposed to 0, 100, and 150 ppm of F were not significantly different to one another.

Table 1. Mean body weight values of the ICGN and ICR mice exposed to the fluoride ion (F) in their drinking water at the end of the 4-week observation period.^a (n= 5 for each group)

| Group | F concentration (ppm) | Body weight (g, mean±SE) | p value ^b |
|-------|-----------------------|--------------------------|----------------------|
| ICGN | 0 | 24.92±2.8 | 0.0001 |
| | 50 | 27.44±1.3 | |
| | 100 | 16.18±0.3*,† | |
| | 150 | 16.74±0.6*,† | |
| ICR | 0 | 39.54±0.8 | 0.826 |
| | 100 | 40.20±0.3 | |
| | 150 | 39.90±1.1 | |

^aWhen a mouse died prior to the end of the 4 week period, the data from the weekly samples taken closest to the day of the death were used; ^bp value calculated by ANOVA. Compared to the 0 ppm of F group: *p<0.05; compared to the 50 ppm of F group: †p<0.05

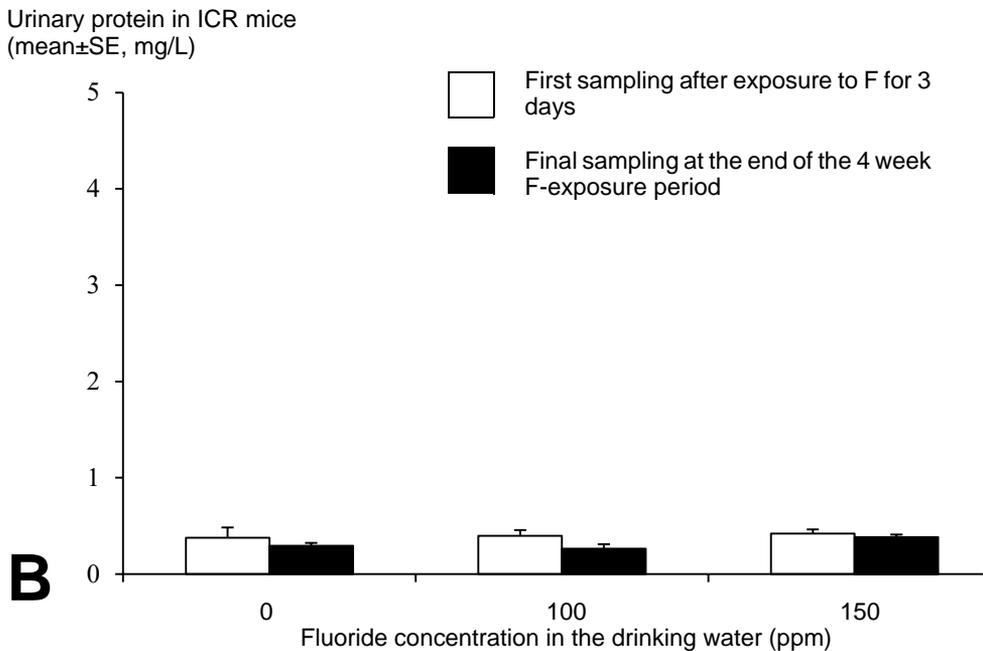
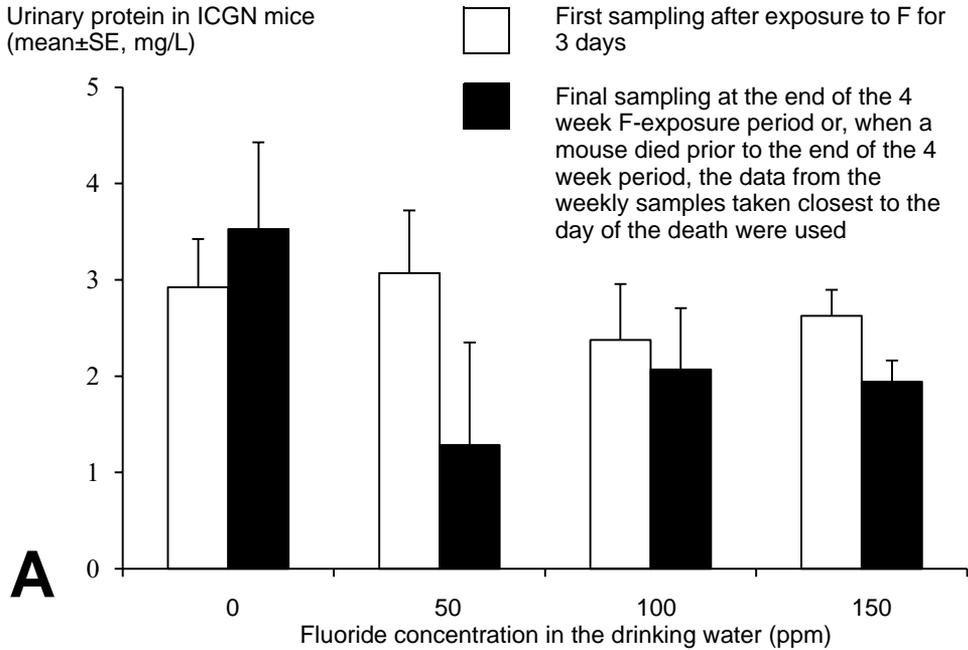
Table 2 shows the mean daily water intake per body weight in the ICGN and ICR mice. The water intake of the ICGN mice exposed to 150 ppm F was markedly lower than those exposed to other concentrations. The water intake of the ICR mice exposed to 150 ppm F was not significantly different to those exposed to the other concentrations.

Table 2. Mean values of the daily water intake per g of body weight (bw) of the ICGN and ICR mice exposed to the fluoride ion (F) in their drinking water at the end of the observation period.^a (n= 5 for each group)

| Group | F concentration (ppm) | Intake of water (mL/g bw/day, mean) |
|-------|-----------------------|-------------------------------------|
| ICGN | 0 | 0.203 |
| | 50 | 0.231 |
| | 100 | 0.196 |
| | 150 | 0.042 |
| ICR | 0 | 0.108 |
| | 100 | 0.143 |
| | 150 | 0.134 |

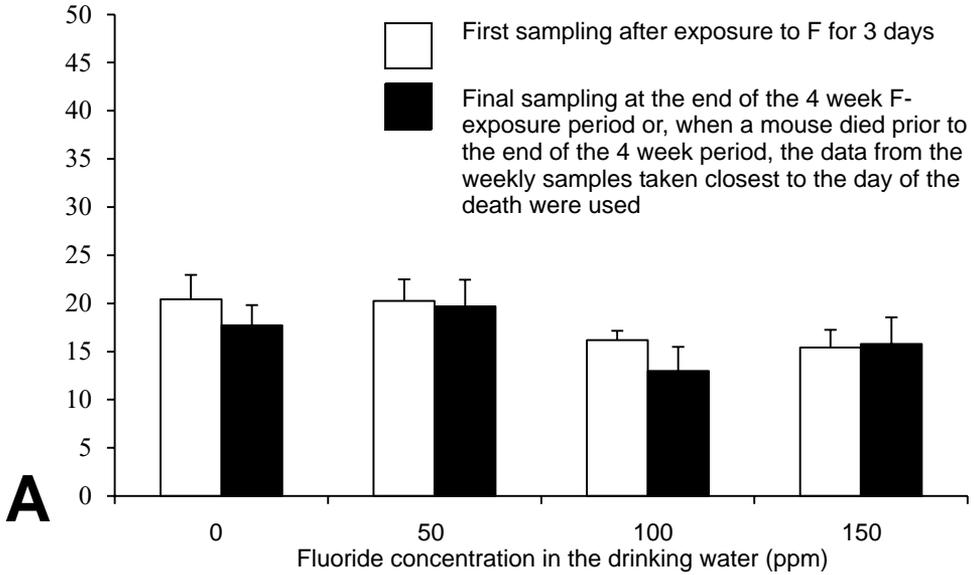
^aWhen a mouse died prior to the end of the 4 week period, the data from the weekly samples taken closest to the day of the death were used.

Figures 2A, 2B, 3A, and 3B show the urinary protein and creatinine concentrations of the ICGN and ICR mice exposed to F in their drinking water.

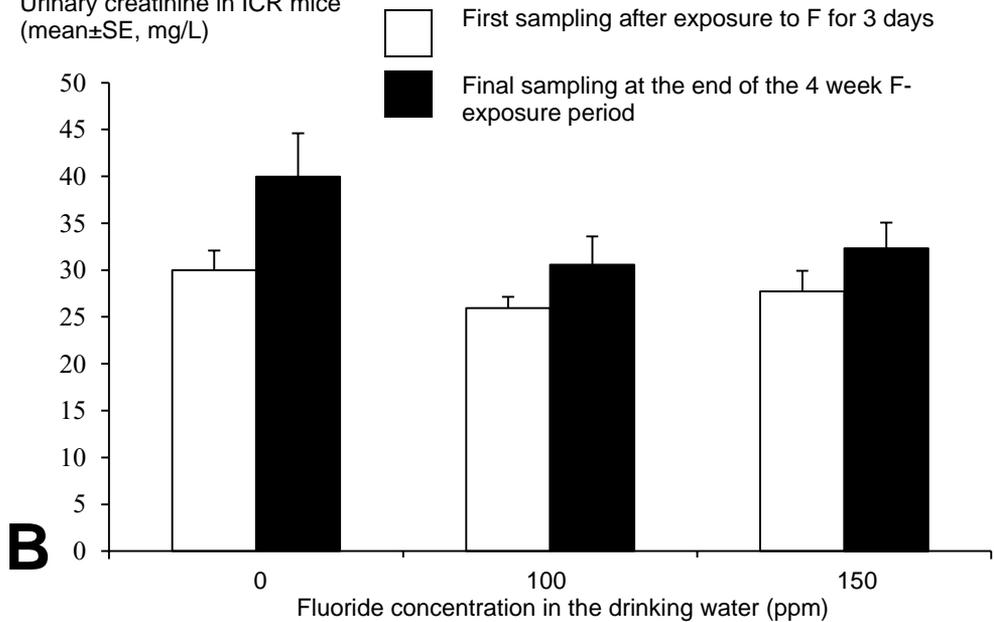


Figures 2A and 2B. The urinary protein concentrations (mean±SE, mg/L) at the time of the first sampling after 3 days and at the end of the 4 week F-exposure period of the (A) ICGN and the (B) ICR mice exposed to various concentrations of the fluoride ion (0, 50, 100, and 150 ppm) in their drinking water. When a mouse died prior to the end of the 4 week period, the data from the weekly samples taken closest to the day of the death were used. n = 5 for each group.

Urinary creatinine in ICGN mice
(mean±SE, mg/L)



Urinary creatinine in ICR mice
(mean±SE, mg/L)



Figures 3A and 3B. The urinary creatinine concentrations (mean±SE, mg/L) at the time of the first sampling after 3 days and at the end of the 4 week F-exposure period of the (A) ICGN and the (B) ICR mice exposed to various concentrations of the fluoride ion (0, 50, 100, and 150 ppm) in their drinking water. When a mouse died prior to the end of the 4 week period, the data from the weekly samples taken closest to the day of the death were used.

There were no significant differences in the urinary protein and creatinine concentration levels at the first sampling and at the end of the observation period in either the ICGN or the ICR mice. Although the urinary protein levels observed in the ICGN mice were higher than those in the ICR mice, the mean protein level in the urine in the 50 ppm ICGN group was lower at the end of sampling compared with the levels at the first sampling. The protein levels in the urine at the first sampling and at the end in the 150 ppm ICGN group were similar. The mean urine creatinine values in the ICGN mice groups were lower than those in the ICR mice groups.

Figures 4A and 4B show the BUN of the ICGN and ICR mice exposed to F in their drinking water. Because all the ICGN mice exposed to 100 and 150 ppm F died prior the end of the 4 week F-exposure period, the data from the weekly samples taken closest to the day of the death were used for their final sampling.

Blood urea nitrogen (BUN) in ICGN mice
(mean±SE, mg/dL)

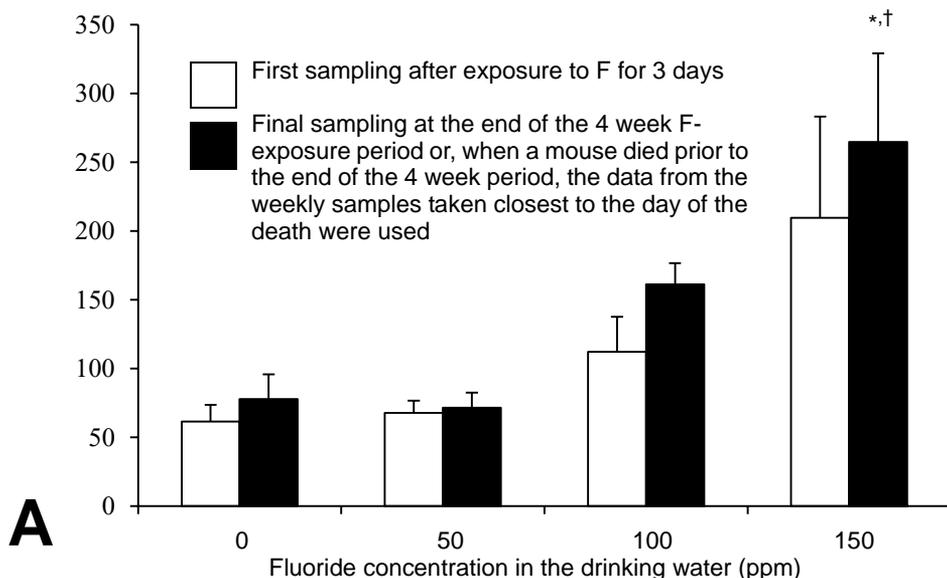


Figure 4A. The blood urea nitrogen (BUN) concentrations (mean±SE, mg/L) at the time of the first sampling after 3 days and at the end of the 4 week F-exposure period of the (A) ICGN mice exposed to various concentrations of the fluoride ion (0, 50, 100, and 150 ppm) in their drinking water. When a mouse died prior to the end of the 4 week period, the data from the weekly samples taken closest to the day of the death were used. n = 5 for each group. The statistical analyses were done using ANOVA followed by a Student-Newman-Keuls test. Compared to the 0 ppm of F group: *p<0.05; compared to the 50 ppm of F group: †p<0.05.

For the ICGN mice, the mean BUN value at the end of sampling for the 150 ppm group was significantly higher than those in the 0 and 50 ppm groups. In addition, combining data from the second and third week of sampling showed

an elevation in the mean BUN value of each group over time (data not shown). The highest values were observed for the 150 ppm group at the end of sampling, while for the ICR mice, the mean BUN value at the first sampling for the 150 ppm group was significantly higher than that in the 0 or 100 ppm groups.

Blood urea nitrogen (BUN) in ICR mice
(mean±SE, mg/dL)

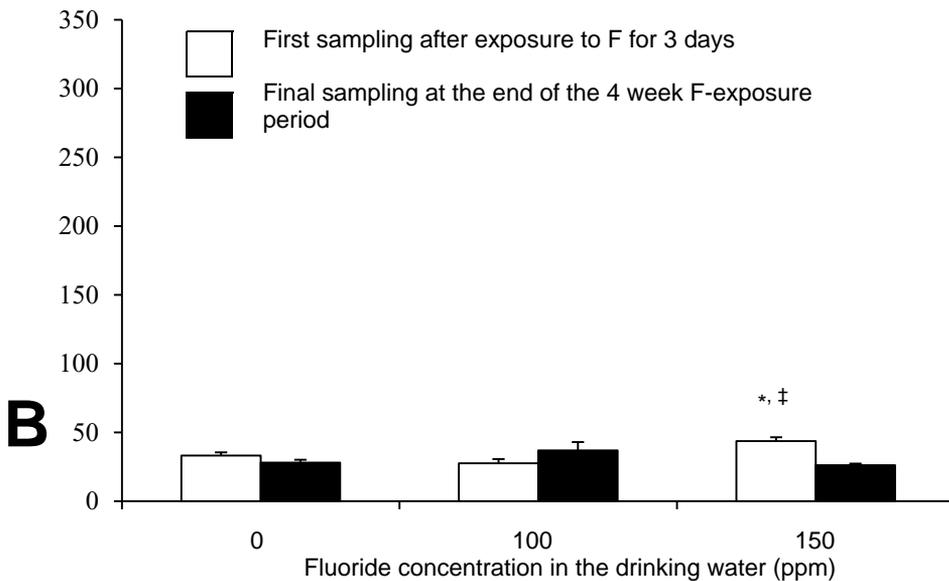
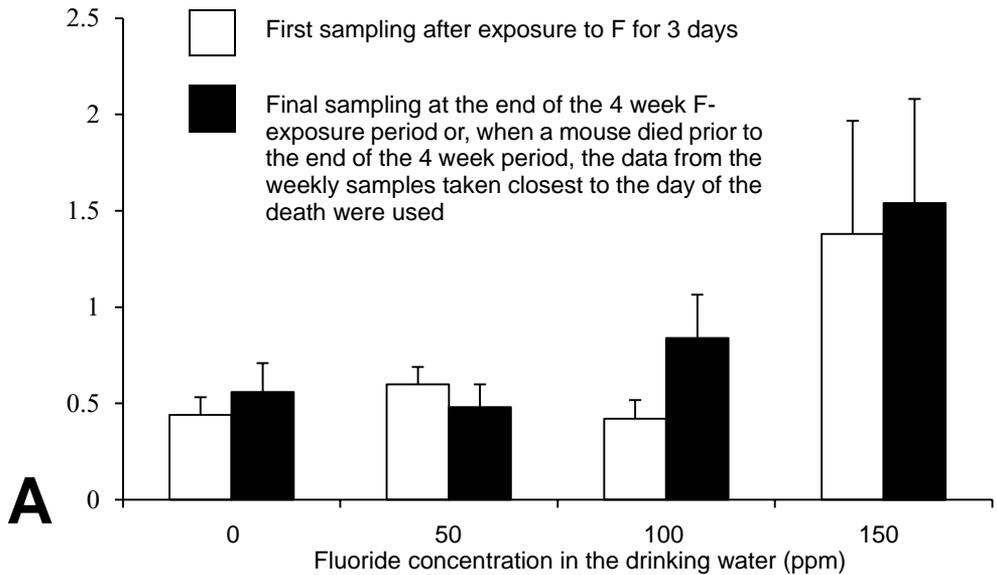


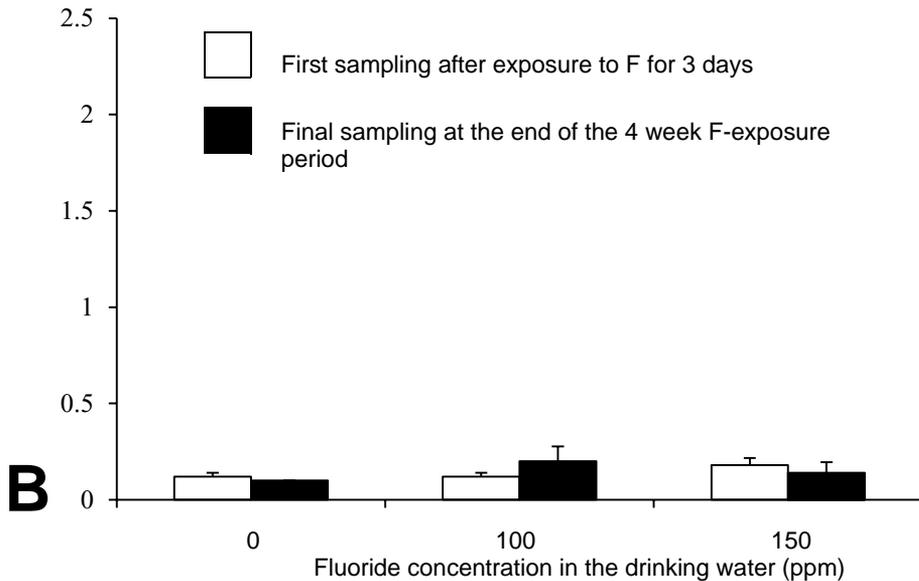
Figure 4B. The blood urea nitrogen (BUN) concentrations (mean±SE, mg/L) at the time of the first sampling after 3 days and at the end of the 4 week F-exposure period of the (B) ICR mice exposed to various concentrations of the fluoride ion (0, 100, and 150 ppm) in their drinking water. n = 5 for each group. The statistical analyses were done using ANOVA followed by a Student-Newman-Keuls test. Compared to the 0 ppm of F group: *p<0.05; compared to the 100 ppm of F group: ‡p<0.05.

Figures 5A and 5B show the serum CRE concentrations of the ICGN and ICR mice exposed to F in their drinking water. Because all the ICGN mice exposed to 100 and 150 ppm F died prior the end of the 4 week F-exposure period, the data from the weekly samples taken closest to the day of the death were used for their final sampling. There were no significant differences in the serum CRE concentrations at the first sampling and at the end of the observation period in either the ICGN or the ICR groups. The values at the end were higher than those at the first sampling in the 100 and 150 ppm ICGN groups.

Serum creatinine (CRE) in ICGN mice
(mean±SE, mg/dL)



Serum creatinine (CRE) in ICR mice
(mean±SE, mg/dL)



Figures 5A and 5B. The serum creatinine (CRE) concentrations (mean±SE, mg/L) at the time of the first sampling after 3 days and at the end of the 4 week F-exposure period of the (A) ICGN mice and the (B) ICR mice exposed to various concentrations of the fluoride ion (0, 100, and 150 ppm) in their drinking water. When a mouse died prior to the end of the 4 week period, the data from the weekly samples taken closest to the day of the death were used. n = 5 for each group.

Table 3 shows the creatinine clearance of the ICGN and the ICR mice exposed to F via their drinking water. Low mean creatinine clearance values were observed among the ICGN groups, and there was no significant difference in creatinine clearance among either the ICGN or the ICR groups. The lowest values at the first sampling and the end of the sampling were observed among the 150 ppm group.

Table 3. Creatinine clearance values (mL/min) of the ICGN and ICR mice exposed to the fluoride ion (F) in their drinking water at the first sampling after 3 days and at the end of the 4-week observation period.^a (n= 5 for each group)

| Group | F concentration (ppm) | Creatinine clearance | | | |
|-------|-----------------------|----------------------|----------------|-------------------|----------------|
| | | First sampling | | End sampling | |
| | | (mean±SE, mL/min) | p ^b | (mean±SE, mL/min) | p ^b |
| ICGN | 0 | 0.131±0.075 | 0.432 | 0.011±0.005 | 0.345 |
| | 50 | 0.101±0.057 | | 0.012±0.006 | |
| | 100 | 0.138±0.066 | | 0.017±0.014 | |
| | 150 | 0.087±0.053 | | 0.003±0.001 | |
| ICR | 0 | 0.337±0.094 | 0.428 | 0.339±0.040 | 0.258 |
| | 100 | 0.214±0.040 | | 0.240±0.058 | |
| | 150 | 0.295±0.068 | | 0.190±0.056 | |

^aWhen a mouse died prior to the end of the 4 week period, the data from the weekly samples taken closest to the day of the death were used; ^bThe data among the groups were compared by ANOVA.

Table 4 shows the mean F concentrations in the urine of the ICGN and the ICR groups. Significant differences were present among both the ICGN and the ICR groups at both the first and the end samplings (p=0.22 and p=0.22; and p=0.0001 and p=0.0001, respectively). For the ICGN mice, at the first sampling, the mean F concentration in the urine of the 100 ppm group was significantly higher (p<0.05) than in the control group. For the ICR mice, the mean F concentration in the urine of the 100 and 150 ppm groups was significantly higher (p<0.0001 and p<0.0001, respectively) than in the 0 ppm group, at both the first sampling and at the end sampling.

Table 4. Urinary fluoride ion concentrations (mg/L) of the ICGN and ICR mice exposed to the fluoride ion (F) in their drinking water at the first sampling after 3 days and at the end of the 4-week observation period.^a (n= 5 for each group)

| Group | F concentration (ppm) | Urinary fluoride ion concentrations | | | |
|-------|--------------------------|-------------------------------------|---------------------|--------------------------|---------------------|
| | | First sampling | | End sampling | |
| | | (mean±SE, mg/L) | p | (mean±SE, mg/L) | p |
| ICGN | 0 | 0.209±0.021 | 0.022 ^b | 0.266±0.062 | 0.022 ^c |
| | 50 | 0.457±0.034 | | 1.128±0.244 | |
| | 100 | 1.064±0.085* | | 1.931±0.455 | |
| | 150 | 0.832±0.359 | | 2.383±1.109 | |
| ICR | 0 | 0.481±0.048 | 0.0001 ^b | 0.511±0.070 | 0.0001 ^b |
| | 100 | 2.401±0.207 [§] | | 2.440±0.179 [§] | |
| | 150 | 2.764±0.376 [§] | | 3.029±0.271 [§] | |

^aWhen a mouse died prior to the end of the 4 week period, the data from the weekly samples taken closest to the day of the death were used; ^bthe data among the groups were compared by ANOVA; ^cthe data among the groups were compared by the Kruskal-Wallis test; *compared to the 0 ppm group by the Student-Newman-Keuls test: *p<0.05; [§]compared to the 0 ppm group by the Student-Newman-Keuls test: [§]p<0.0001.

DISCUSSION

F is an environmental pollutant that contaminates ground water. Given that F is excreted by the kidneys, the toxic effect of F is increased in animals with impaired renal function.^{8,11,15} It has been reported that all ICGN mice exposed to 150 ppm F in their drinking water died within four weeks.^{8,11} The mean BUN and CRE values in the ICGN mice exposed to 150 ppm F in their drinking water were significantly higher than those in the control mice.⁸ However, in our literature review, we found no information on the effects of F in animals with impairments in renal function and the creatinine clearance.

In the present study, the lethal effect of ≥100 ppm F in drinking water on ICGN mice with impaired renal function was demonstrated. However, there was no difference in body weight among the ICR groups. These results clearly show the serious toxic effects of ≥100 ppm F in the drinking water of mice with an impaired kidney function. Previously, it has been demonstrated that ICGN mice exposed to

100 ppm F in drinking water did not die after a four week exposure.^{8,11} The discrepancies in the lethality of 100 ppm F in drinking water among the studies may be due to differences in the severity of the renal damage in the mice among the studies. In addition, in the present study, the stress caused by metabolic cages may have attributed to the lethal effect of 100 ppm F.¹³

The water intake of the 150 ppm ICGN group was low, and this may have caused the rapid deterioration in renal function resulting in a decline in the general condition of these mice. The water intake of the 150 ppm ICR group was not different from those of other groups. The urinary protein levels in the ICGN mice were generally high because of their glomerulonephritis. The mean protein level in the urine in the 50 ppm ICGN group at the end was quite low, which suggested they were in poor general condition, while the protein levels in the urine at the first sampling and at the end sampling in the 100 and 150 ppm ICGN groups were similar. Before a deteriorating condition can be identified by lower protein levels in the urine, mice exposed to 100 or 150 ppm F might die rapidly.

For the ICGN mice group, significantly higher mean BUN values were observed at the end in the 150 ppm group compared to the control and 50 ppm groups. Also, exposure to 100 and 150 ppm F in the drinking water induced an elevated BUN three days after the exposure and afterwards. The rapid increase in BUN observed at the first sampling and afterwards might be due to an acute impairment of renal function due to F toxicity among the ICGN mice. A significant increase in BUN was observed around 10 days after 100 ppm F exposure via drinking water among ICGN mice.⁸ In the current study, the glomerulonephritis might have already been deteriorating prior to the time of the first sampling after 3 days of exposure resulting in the earlier increase in BUN.

In the ICR mice group, a significantly higher mean BUN value in the 150 ppm group compared to the control group at the first sampling was observed. While this might be a toxic effect of F, the BUN in the 150 ppm group was lower at the end sampling, suggesting that the effect might have only been temporary.

There was no significant difference in the creatinine concentration in the urine and serum at the first sampling and at the end sampling among the ICGN and the ICR groups. There was also no significant difference in the creatinine clearance at the first sampling and at the end sampling. However, the mean creatinine clearance of the ICGN mice was markedly lower than that of the ICR mice. In the ICGN mice, the glomerular filtration was lower over the observation period although it was not decreased by F exposure.

For the ICGN mice, the F concentration in the urine at the first sampling of the 100 ppm group was significantly higher than that of the 0 ppm group. For the ICR mice, the F concentrations in the urine of the 100 and 150 ppm groups at the first sampling and the end sampling were significantly higher than that of the 0 ppm group. The higher F concentrations in the urine from the ICGN group exposed to 100 or 150 ppm were due to the higher serum F levels in mice that had impaired kidney function.⁸

CONCLUSIONS

In conclusion, these results clearly show the serious toxic effect of ≥ 100 ppm F in the drinking water for mice with impaired kidney function. In particular, the early increase in the BUN of the ICGN mice provides an adequate index of the deterioration of kidney function caused by F.

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