

ACUTE RENAL DAMAGE DOSE RESPONSE IN RATS TO INTRAVENOUS INFUSION OF SODIUM FLUORIDE

Tomotaro Dote,^a Koichi Kono, Kan Usuda,
Hiroyuki Nishiura, Teruaki Tagawa
Osaka, Japan

SUMMARY: Rapid acute renal injury in Wistar rats from fluoride (F) following single-dose NaF infusions of 0 mg F (control), 1 mg F (Group 1), 2 mg F (Group 2), and 3 mg F (Group 3) was assessed by examining three collections of bladder urine at 2-h intervals for six hours after the infusions. The urinary parameters investigated were: urine volume, excretion of F, creatinine, and α -glutathione S-transferase (α -GST), and the activity of N-acetyl- β -D-glucosamidase (NAG). Compared with the control, the urine volume increased (polyuria) in Group 1 but decreased in groups 2 and 3. F excretion increased in groups 1 and 2 but was lower in Group 3 than in Group 1. In Group 3, creatinine excretion decreased compared with the control, but the activities of α -GST and NAG steadily increased in Group 3. The decreases in F and creatinine excretion in Group 3 are attributable to glomerular dysfunction, and the increases in α -GST and NAG activities in Group 3 indicate acute proximal renal tube injury. The kidney toxicity of intravenous administration of NaF to laboratory rats was thus dose related, and an infusion of 3 mg F was sufficient to cause acute renal dysfunction.

Keywords: Acute renal damage, Creatinine excretion, Fluoride dose response, Fluoride excretion, α -Glutathione S-transferase, Intravenous NaF, N-acetyl- β -D-glucosamidase, Rat kidney dysfunction, Urinary fluoride.

INTRODUCTION

Fluoride (F) products of various kinds occur and are used widely in industry and medicine. Because absorbed F is eliminated mainly through the kidney, urinary F is generally used to monitor F intake in workers exposed to F. However, the kidney is also a primary target organ for F toxicity. Whenever renal function is significantly impaired, urinary F excretion decreases, and serum F concentrations increase.¹ Thus urinary F dose not always correlate with the level of F exposure or intake. The purpose of this study was to use urinary parameters to determine the relationship between F dose and acute kidney intoxication in rats from single intravenous infusions of sodium fluoride.

MATERIALS AND METHODS

Twenty-eight 11-week-old SPF Wistar male rats (Japan SLC, Hamamatsu, Shizuoka, Japan) weighing 340-350 g were fed a standard laboratory diet (MM-3; Funabashi Farms, Funabashi, Chiba, Japan). The animals were allowed to ingest food and drink tap water (0.1 ppm F) *ad libitum*. They were housed in automatic flush cages (3 or 4 rats per cage) in a temperature-controlled room at $22.5 \pm 2^\circ\text{C}$ with a 12-hr/day illumination cycle from 0800 to 2000 hours and at 50-60% relative humidity. After observation for one week to

^aFor correspondence: Department of Hygiene and Public Health, Osaka Medical College, 2-7 Daigakumachi, Takatsuki, Osaka 569, Japan.
E-mail: hyg011@art.osaka-med.ac.jp

verify their good health, the animals were divided equally into four groups. Jugular veins and bladders were exposed under peritoneal nembutal anesthesia.

Intravenous infusions were administered over a 30-min period with a micro feeder (Model JP-V, Furue Science Co. Ltd). Each animal in the control group was administered 16 mL of physiological saline (0.9 M NaCl) only. The animals in the other three groups were given saline and then saline containing NaF at a concentration of 1.000 mg F/mL as follows. Group 1 was given 15 mL of saline and then 1 mL of NaF solution (2.86 mg F/kg body weight). Group 2 was administered 14 mL saline and then 2 mL of NaF solution (5.71 mg F/kg), and Group 3 was given 13 mL of saline and then 3 mL NaF solution (8.57 mg F/kg). After the infusions, urine was collected three times from each bladder at 2-hr intervals. Indwelling catheters (18 G indwelling needle: Termo Co. Ltd) were stuck into bladders with an inclination of 20 degrees. All urine soon drained into the sampling tube without remaining in the bladder to make the first, second and third 2-hr samples reliably comparable.

The urinary parameters measured were: urine volume and the excretion of F, creatinine, N-acetyl- β A-D-glucosamidase (NAG), and α -glutathioneS-transferase (α -GST).

Measurement of urinary parameters: F was measured with a F electrode using an Orion Model EA-940 ion analyzer in a buffer to adjust ionic strength. creatinine was measured by the Jaffe method using the Wako creatinine test (Wako Pure Chemical Ind, Osaka, Japan) and with a Hitachi model 200-20 spectrophotometer at 520 nm. α -GST was measured by the enzyme-linked immunosorbent assay (ELISA) method with Rat Nephkit-Alfa (Biotrin International, Dublin, Ireland) using a Bio-Rad P-450 microplate reader at 450 nm with 630 nm as a reference. NAG was measured by the MCP-NAG (Sodio-m-cresolsulfonphthaleinyl-N-acetyl- β -D-glucosaminide) method using the NAG test (Shionogi Co, Osaka, Japan).² Total activities were calculated with urine volume and NAG concentration (U/L) to consider variation of urine volume.

Statistical analysis: StatView version 5.0 (SAS Institute Co, Inc. USA) was used to perform a one-way ANOVA to test the significance of the difference of the total doses of each urinary parameter. The Bonferroni-Dunn test was applied to determine the significance of the differences among three groups.³ The significant time-dependencies among three groups were tested by repeated ANOVA tests. Values were considered statistically significant at $p < 0.05$. The correlation among parameters was examined by matching the duration of sampling. A value of $p < 0.05$ was considered statistically significant. The values are reported as the mean \pm standard deviation. All experiments described in this paper were performed with adherence to the experimental animal use guidelines of the Japanese Association for Laboratory Animal Science.

RESULTS

Urine volume increased in Group 1 compared with the control ($p < 0.01$). It decreased in Group 2 ($p < 0.05$) and Group 3 ($p < 0.01$). Significant time-

dependencies were observed between the control and groups 2 and 3 ($p < 0.01$), between groups 1 and 3 ($p < 0.01$) and between groups 2 and 3 ($p < 0.05$) (Figure 1). The level of F excretion for the control was very low.

Figure 1. Urine volume after F infusion

Mean \pm SD (N=7) * $p < 0.05$. ** $p < 0.01$.

F excretion in Group 2 increased slightly compared with Group 1. No significant increase in F excretion was observed in Group 3 compared with Group 1 (Figure 2). Creatinine excretion decreased in Group 3 compared with the control and Group 1 ($p < 0.01$). A significant time-dependency was observed between the control and Group 3 ($p < 0.05$) (Figure 3). A slight increase in α -GST excretion was observed in Group 2. There was a significant increase in α -GST level in Group 3 compared with the control and Group 1 ($p < 0.01$). A significant time-dependency was observed between the control and Group 3 ($p < 0.01$) (Figure 4). NAG excretion showed a tendency to increase in Group 3. A significant time-dependency was observed between the control and Group 3 ($p < 0.05$) (Figure 5). Figure 6 shows the relationship between creatinine and F in Group 1 ($r = 0.838$, $p < 0.01$). There were no close relationships between F excretion and creatinine excretion in groups 2 and 3. Figure 7 shows the relationship between NAG and α -GST in Group 3 ($r = 0.813$, $p < 0.01$).

Figure 2. F excretion after F infusion

Mean \pm SD (N=7).

Figure 3. Creatinine (Cr) excretion after F infusion

Mean \pm SD (N=7). **p<0.01.

Figure 4. α -GST excretion after F infusion

Mean \pm SD (N=7). **p<0.01.

Figure 5. NAG activity after F infusion

Mean \pm SD (N=7).

Figure 6. Correlation between creatinine excretion and F excretion in Group 1 animals

Creatinine excretion (μg).

Figure 7. Correlation between NAG activity and α -GST excretion in Group 3 animals

NAG activity (X 1/10 U).

DISCUSSION

It has been found previously that F clearance is dependent on glomerular filtration rate and that there is a direct correlation of F clearance with urinary flow.¹ Here a close relationship between F excretion and creatinine excretion was observed in Group 1, though there were no close relationships between F excretion and creatinine excretion in groups 2 and 3. Creatinine excretion, NAG and α -GST values showed that nephrotoxicity was barely evident in Group 1. Polyuria in laboratory rats after oral administration of F has also been reported,⁴ and F-induced polyuria was observed here in Group 1. Since F is excreted mainly from the kidney, harmful effects of F retention are directly related to renal function.⁵ Urinary excretion of F and creatinine decreases if renal function is reduced, and F clearance depends on creatinine clearance.⁶ Decrease of both urine volume and creatinine excretion along with reduced excretion of F in Group 3 suggested acute dysfunction of glomerulus caused by exposure to the high level of F.

NAG is also a useful nephrotoxic indicator.⁷ NAG activity is at its maximum in the proximal convoluted tubule (PTC S1 segment).⁸ α -GST is a suitable marker for chemically-induced tubular damage,⁹ particularly in the S3 segment of the proximal tubule.^{10,11} It has been found that increases in α -GST and NAG excretion indicate acute proximal tubular injury,¹² and here a significant correlation between NAG and α -GST suggested that the damage to PTC or S3 was concomitant. Thus the strong increased activity relationship of NAG with α -GST in Group 3 indicated damage to S1 and S3. These results showed that acute exposure to the 3-mg dose of F in Group 3 (8.57 mg F/kg) damaged renal tissue and caused renal dysfunction. Such renal toxicity, however, was not observed in Group 1 (2.86 mg F/kg). A dose-response relationship has therefore been demonstrated between intravenous administration of F and kidney damage in laboratory rats. Since acute renal dysfunction decreases F excretion, some other renal markers such as NAG and α -GST are also necessary for monitoring F exposure and intake.

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