

Time-dependent Changes of Blood Parameters and Fluoride Kinetics in Rats after Acute Exposure to Subtoxic Hydrofluoric Acid

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Abstract: Time-dependent Changes of Blood Parameters and Fluoride Kinetics in Rats after Acute Exposure to Subtoxic Hydrofluoric Acid: Masafumi IMANISHI, et al. Department of Hygiene and Public Health, Osaka Medical College—**Objectives:** In our previous study, we reported that even a sublethal dose of hydrofluoric acid (HFA) could cause acute toxic effects 60 min after intravenous injection. This study was designed to investigate the time- and dose-dependent changes associated with these disorders. The serum fluoride (F) kinetics are also considered in the discussion of the relationship between the concentrations of serum F and the disorders. **Methods:** Rats were injected with HFA (1.6 or 9.6 mg/kg body weight) for the dose-response relationship study. For each dose, the rats were assigned to one of seven groups. Blood samples of the 0-min group were obtained from the carotid artery prior to injection as a control. The other six groups were labeled according to sampling times (5, 10, 30, 60, 120 and 300-min) in the time-dependent study. **Results:** The 1.6 mg/kg dose decreased the ionized calcium (Ca^{2+}) level significantly after 30 min, and it also decreased the total calcium (Ca) level after 300 min. The 9.6 mg/kg dose rapidly worsened renal dysfunction after 60 min. It increased the serum potassium level after 60 and 120 min and it decreased Ca and Ca^{2+} levels until 300 min. Although there was respiratory compensation, the base excess and HCO_3^- level and had not completely recovered by 300 min. **Conclusions:** Even low exposure to HFA caused renal dysfunction, and electrolyte abnormalities and metabolic acidosis lasted for several hours in rats. Therefore, persons involved in HFA accidental exposure should be closely monitored over time, even if the exposure is less than the sublethal dose.

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Key words: Acute exposure, Fluoride kinetics, Hydrofluoric acid, Hyperkalemia, Hypocalcemia, Renal failure

Hydrogen fluoride (HF) is a colorless gas that is highly corrosive and hydrophilic. Liquefied HF is a strong protonic acid. The dilute solution is called hydrofluoric acid (HFA), which is a strong inorganic acid. HFA corrodes glass and metals except gold and platinum. It is used widely in many industrial processes, such as in the washing of semiconductors, polishing of metals, etching and frosting of glass, and in cleaning solutions and laundry powder. HFA can cause severe dermal burns. HFA penetrates the skin rapidly and enters the systemic circulation. HFA exposures have led to systemic complications owing to the potential of fluoride ions (F) to penetrate the skin¹. There are many clinical reports on cardiac dysfunction a few hours after accidental HFA exposure^{2–5}. Furthermore, there have also been many reports on the acute lethal effects of inhaled HFA in relation to occupational accidents^{6, 7}. We also reported that a worker was severely sprayed on his face during a HFA operation and cardiopulmonary arrest occurred after about half an hour^{2, 8}. The serum HFA level rapidly increases upon inhalation compared with dermal exposure due to the direct absorption of HFA into the blood through alveolar-capillary vessel walls. In our previous study, the 24-h i.v. lethal dose (LD) was determined for the rat. The dose-effect relationship was also investigated 60 min after i.v. administration at the maximal dose corresponding to the sublethal dose of HFA (LD_{50}) to model the rapid absorption of HFA into the blood, such as upon acute inhalation exposure. This dose could cause acute renal dysfunction, electrolyte abnormalities and metabolic acidosis after 60 min⁹. However, the early changes associated with harmful effects would rapidly fluctuate owing to the combined effects of dose and time. Therefore, this study investigated the acute-phase responses after a sublethal dose of HFA. Furthermore, the F kinetics were

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also studied as serum metabolism of HFA to consider the relationship between the concentrations and toxic effects.

Materials and Methods

Animals

Ten-week-old specific-pathogen-free male Sprague-Dawley rats weighing 290–300 g were obtained from Japan SLC (Shizuoka, Japan). The animals had free access to rat chow (Funabashi Farm MM-3; Chiba, Japan) and tap water and were housed in a separate room at a constant temperature ($22.0 \pm 1.0^\circ\text{C}$) under a 12-h light/dark cycle. All aspects of this study were conducted under the guidelines of the Osaka Medical Ethical Association for Accreditation of Laboratory Animal Care (approval number (date); 20020 (July 11, 2008)).

Chemical

HFA (concentration 46%) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The chemical was dissolved or diluted in distilled water.

Experimental design

1) Dose-related differences

Eighty-four rats were divided into two dose groups, 1.6 mg/kg and 9.6 mg/kg body weight, which were chosen to compare dose-related differences. These doses were the minimum and the maximum doses, respectively, in our previous study⁹. All the rats were anesthetized with pentobarbital. HFA solutions were rapidly injected through the tail vein.

2) Time-dependent changes after acute exposure

For each dose, each of the forty-two rats was assigned to one of seven groups ($n=6$). Blood samples of the 0-min group were obtained from the carotid artery prior to injection as a control. The other six groups were labeled according to sampling time. Blood samples were obtained from the carotid artery at 5, 10, 30, 60, 120 and 300 min after the bolus injections.

3) Serum parameters

The following serum parameters were measured according to standard protocols recommended by assay suppliers. Kidney function was determined according to blood urea nitrogen (BUN, urease-glutamic dehydrogenase method) and creatinine (Cr, Jaffe method) levels. Other harmful systemic effects, such as acute electrolyte disturbance and metabolic acidosis, were determined on the basis of the following parameters: sodium (Na, Na⁺-selective electrode method), potassium (K, K⁺-selective electrode method), chlorine (Cl, Cl⁻-selective electrode method), total calcium (Ca, OCPC method), ionized calcium (Ca²⁺, Ca²⁺-selective electrode method), and phosphate (P, molybdic acid UV method). The levels of Ca \times P product, magnesium (Mg, xylydyl blue colorimetry), pH, PO₂, PCO₂, HCO₃⁻, base excess (BE) (288 Blood Gas System; Bayel, Osaka, Japan), and ionized fluoride (F, F⁻-selective electrode method) were

also determined.

4) Kinetic analysis of F

The serum concentration-time profiles of F measured after the single injection were analyzed by compartmental modeling with a nonlinear least-squares regression program (WinNonlin; Pharsight Corp., Cary, NC). The WinNonlin program was used to calculate model predictions using standard equations¹⁰. The data were matched to one-, two- and three- compartment models by the serum F concentration predicted using the model. A noncompartmental modeling method was applied to determine the area under the curve values (AUC_{0→300} and AUC_{0→∞}).

Statistical analysis

Data are expressed as mean \pm SD. Overall differences between each group and the control group (time=0) were analyzed by one-way ANOVA. If significance was shown, Fisher's protected least significant difference (LSD) test was applied. All the statistical analyses were performed using SPSS 11.0J software. $P<0.05$ was considered significant for all analyses. Parameters in each group were compared with those of the control group.

Results

The changes in the 1.6 and 9.6 mg/kg groups are shown on the left and right sides of all the figures, respectively. The BUN and Cr levels after the intravenous bolus injection of HFA are shown in Fig. 1. In the 1.6 mg/kg group, the BUN and Cr levels showed no remarkable changes in any of the time groups compared with the 0-min group. In the 9.6 mg/kg group, the BUN and Cr levels time-dependently increased in the 60-, 120- and 300-min groups. The changes in the K and Ca²⁺ levels are shown in Fig. 2. In the 1.6 mg/kg group, the K level showed no remarkable changes in any of the time groups compared with the 0-min group. The Ca²⁺ level significantly decreased in the 30-min group. In the 9.6 mg/kg group, the K level increased in the 60- and 120-min groups, and the Ca²⁺ level decreased in the 10-, 30-, 60-, 120- and 300-min groups. The changes in the Ca and P levels are shown in Fig. 3. In the 1.6 mg/kg group, the Ca level significantly decreased in the 300-min group compared with the 0-min group. The P level increased in the 30- and 60-min groups, and it tended to decrease in the 120- and 300-min groups. In the 9.6 mg/kg group, the Ca level decreased in all the time groups, particularly in the 60-min group. The P level increased in the 120- and 300-min groups. The Ca \times P product increased in the 30- and 60-min groups in the case of the 1.6 mg/kg dose, whereas it decreased in the 5-, 10- and 30-min groups in the case of the 9.6 mg/kg dose. The Na, Cl and Mg levels did not change considerably (data not shown). The changes in the pH are shown in Fig. 4. In the 1.6 mg/kg group, the pH and BE showed no considerable changes in any of the time groups. In the 9.6 mg/kg group, the pH decreased in the 5-min group, while the BE decreased in all of the time groups.

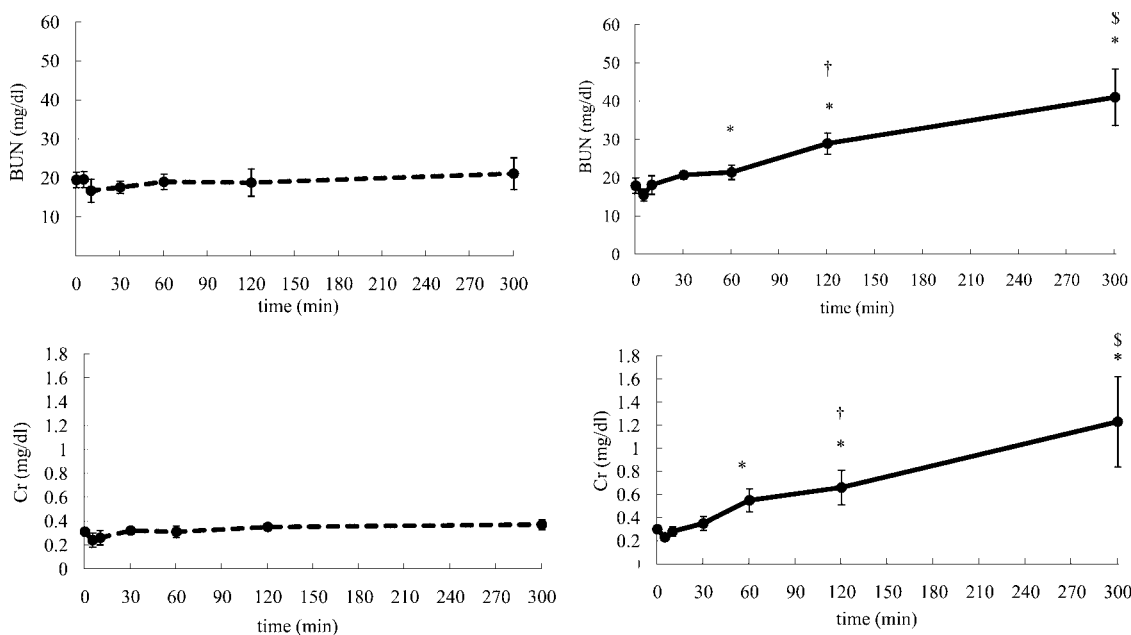


Fig. 1. BUN and serum creatinine (Cr) levels after a single intravenous injection of HFA (left, 1.6 mg/kg; right, 9.6 mg/kg). Mean \pm SD; n=6 Fisher's protected LSD; * p <0.05 vs. time=0, † p <0.05 vs. time=60, § p <0.05 vs. time=120.

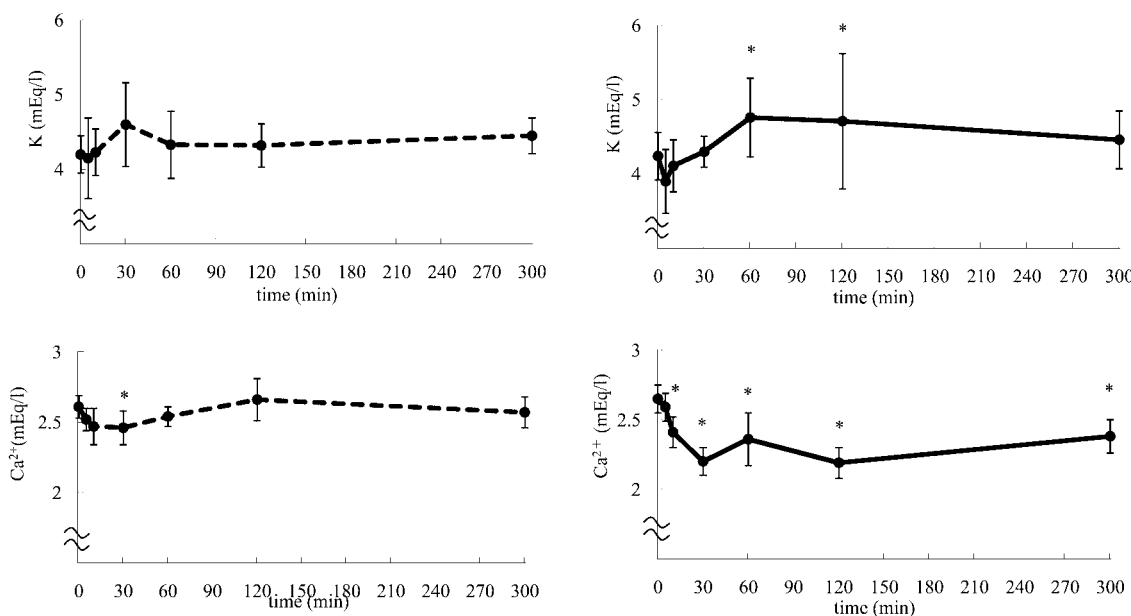


Fig. 2. Serum potassium (K) and ionized calcium (Ca^{2+}) levels after a single intravenous injection of HFA (left, 1.6 mg/kg; right, 9.6 mg/kg). Mean \pm SD; n=6 Fisher's protected LSD; * p <0.05 vs. time=0.

The changes in the PO_2 and PCO_2 are shown in Fig. 5. In the 1.6 mg/kg group, the PO_2 and PCO_2 showed no remarkable changes in any of the time groups. In the 9.6 mg/kg group, the PO_2 significantly increased in the 30- and 120-min groups. The PCO_2 decreased in the 30-, 60- and 120-min groups. The changes in the HCO_3^- concentration

are shown in Fig. 6. In the 1.6 mg/kg group, the HCO_3^- concentration showed no remarkable changes in any of the time groups. In the 9.6 mg/kg group, HCO_3^- concentration decreased in the 30-, 60-, 120- and 300-min groups compared with the 0-min group. The changes in the F concentration are shown in Fig. 7. In the 1.6 and 9.6 mg/kg groups, the

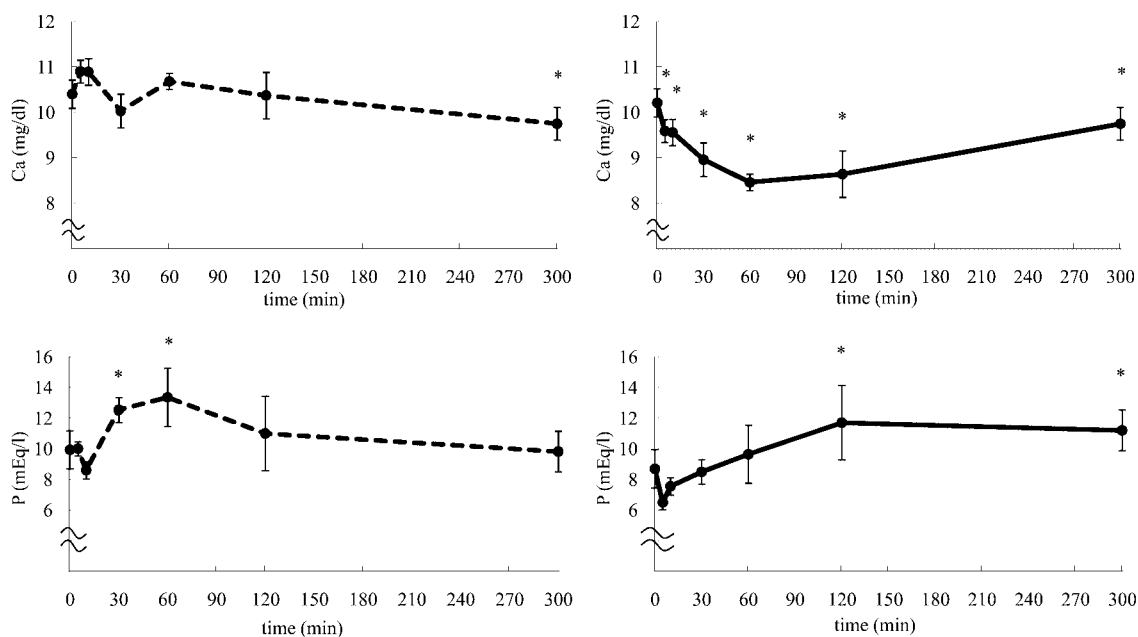


Fig. 3. Serum total calcium (Ca) and phosphate (P) levels after a single intravenous injection of HFA (left, 1.6 mg/kg; right, 9.6 mg/kg). Mean \pm SD; n=6 Fisher's protected LSD; * p <0.05 vs. time=0.

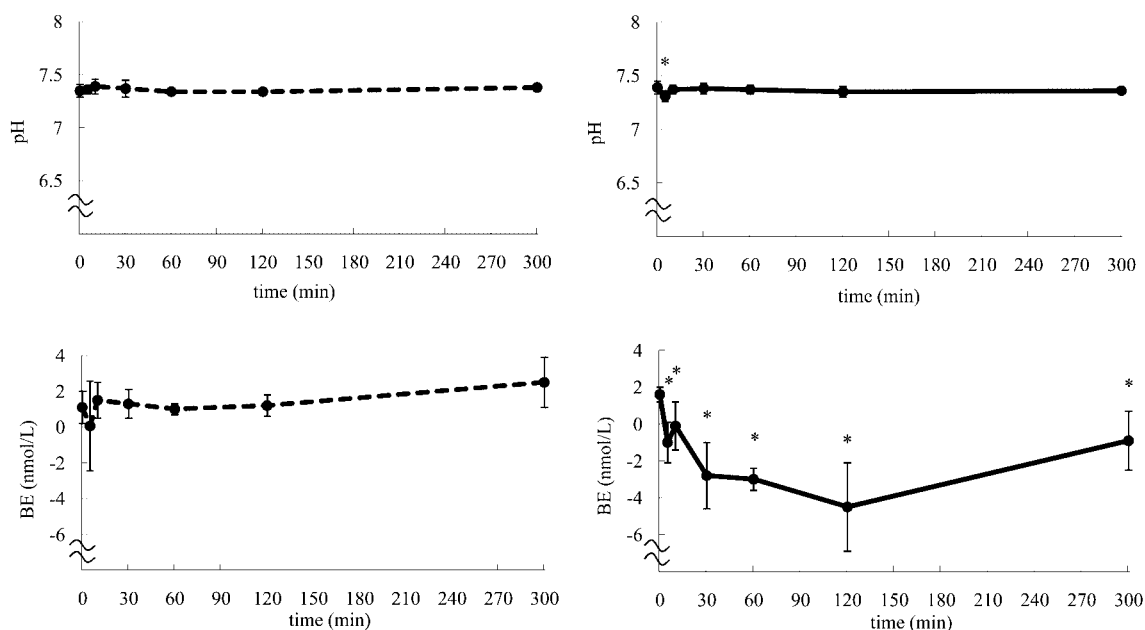


Fig. 4. Serum pH and base excess (BE) after a single intravenous injection of HFA (left, 1.6 mg/kg; right, 9.6 mg/kg). Mean \pm SD; n=6 Fisher's protected LSD; * p <0.05 vs. time=0.

mean serum F concentration rapidly increased in the 5-, 10-, 30- and 60-min groups, but it showed no apparent differences in the 120- and 300-min groups compared with the 0-min group. The serum concentration-time profile of F in the 1.6 and 9.6 mg/kg groups best fitted the one-compartment model and two-compartment model, respectively. The means and SD of $AUC_{0 \rightarrow 300}$ in the 1.6 and

9.6 mg/kg groups were 179 ± 25.8 and 989 ± 186 , respectively. The means and SD of $AUC_{0 \rightarrow \infty}$ in the 1.6 and 9.6 mg/kg groups were 240 ± 132 and 991 ± 186 , respectively. The means and SD of maximum concentration (C_{max}) in the 1.6 and 9.6 mg/kg groups were 5.17 ± 0.45 and $25.7 \pm 3.32 \mu\text{g/ml}$, respectively. The means and SD of the half-life of F ($T_{1/2}$) in the 1.6 mg/kg group were $16.7 \pm$

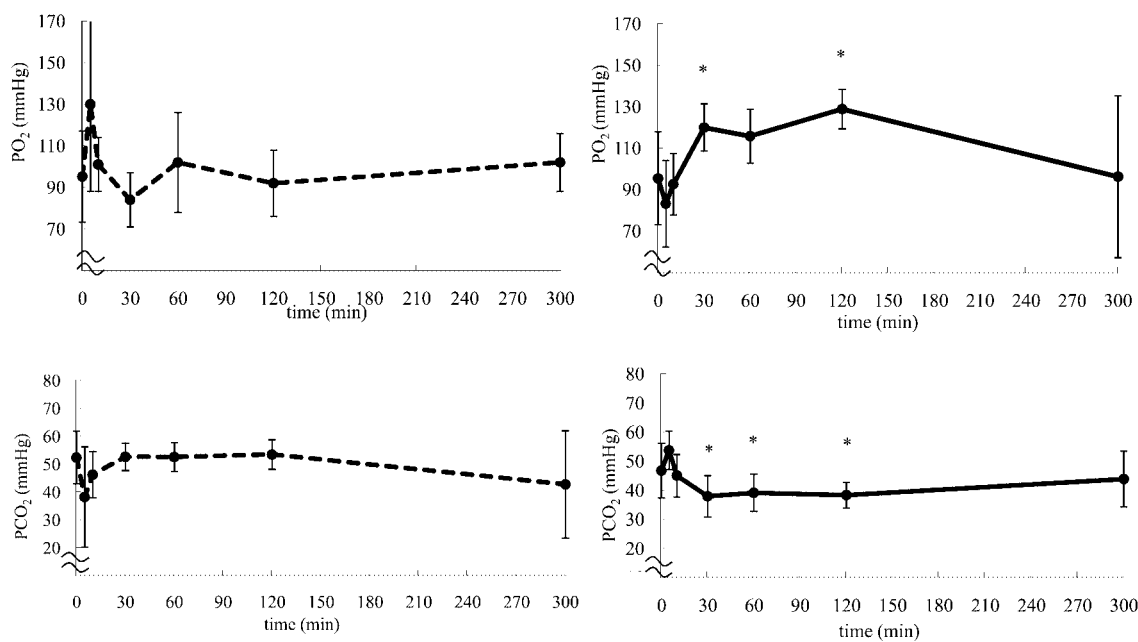


Fig. 5. PO₂ and PCO₂ after a single intravenous injection of HFA (left, 1.6 mg/kg; right, 9.6 mg/kg). Mean ± SD; n=6 Fisher's protected LSD; *p<0.05 vs. time=0.

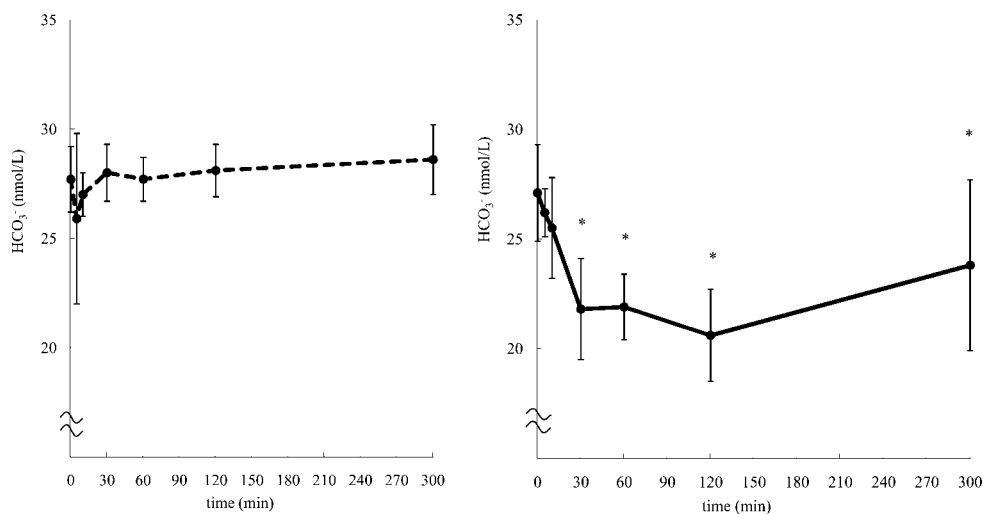


Fig. 6. HCO₃⁻ levels after a single intravenous injection of HFA (left, 1.6 mg/kg; right, 9.6 mg/kg). Mean ± SD; n=6 Fisher's protected LSD; *p<0.05 vs. time=0.

3.0 min. The means and SD of the half-life of F in the α phase (T_{1/2α}) and the β phase (T_{1/2β}) in the 9.6 mg/kg group were 19.6 ± 4.6 and 78.6 ± 6.4 min, respectively.

Discussion

In the 9.6 mg/kg group, the BUN and Cr levels significantly increased in the 60-min group in this study. It was reported that intravenous administration of HFA (3.2, 6.4, and 9.6 mg/kg) significantly increases the BUN and Cr levels after 60 min in the rat, suggesting that acute

glomerular injury occurs at HFA concentrations higher than 3.2 mg/kg after 60 min⁹⁾. The BUN and Cr levels increased more in the 120-min group, and those of the 300-min group were more than twice those of the 0-min group in the case of 9.6 mg/kg dose. It was confirmed in this study that renal disorder appears more marked from 120 to 300 min, since the time-dependent increases of BUN and Cr indicated the rapid worsening of renal dysfunction. We suggest that the progressive renal injury was caused by F toxicity, because it was reported that the infusion of

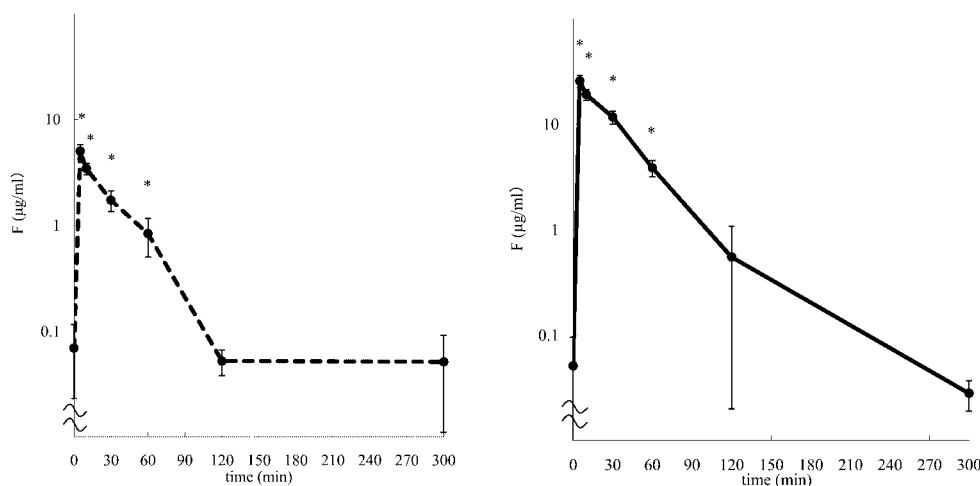


Fig. 7. Serum concentration of ionized fluoride (F) after a single intravenous injection of HFA (left, 1.6 mg/kg; right, 9.6 mg/kg). Mean \pm SD; n=6 Fisher's protected LSD; * p <0.05 vs. time=0.

approximately 9 mg/kg F in NaF is sufficient to cause acute glomerular dysfunction and proximal renal tubular injury within 6 h in rats¹¹). Furthermore, the renal dysfunction could have been induced by cardiac dysfunction such as organic injury caused by toxic myocarditis. It was reported that myocardial injuries are pathologically found within several hours of exposure to HFA in clinical cases^{4,12}). The K levels showed no remarkable changes in the case of 1.6 mg/kg dose. On the other hand in the case of the 9.6 mg/kg dose, the K levels increased in the 60- and 120-min groups. We previously reported that electrolyte abnormalities and metabolic acidosis were aggravated by acute renal damage and the K level increased at HFA concentrations higher than 6.4 mg/kg, 60 min after intravenous injection⁹). It was confirmed in this study that the serum concentration of K is abnormally high 60 to 120 min after a single dose of 9.6 mg/kg HFA. However the K concentration had recovered at 300 min despite the deterioration of renal function. Therefore, K level might also be increased by the disruption of extrarenal mechanisms such as ion channels¹³). As a mechanism of hyperkalemia caused by F toxicity, two *in vitro* studies showed that K effluxes from erythrocytes that are in contact with F, and F increases the intracellular Ca^{2+} level, which is considered to trigger the activation of Ca^{2+} -dependent K channels producing K effluxes^{14,15}). Therefore, we suggest that the K concentration can recover after 120 min due to reduction of the F concentration. The Ca^{2+} level decreased in the 30-min group in the case of the 1.6 mg/kg dose, and it showed apparent decreases in all the time groups in the case of the 9.6 mg/kg dose. The Ca level significantly decreased in the 300-min group in the case of the 1.6 mg/kg dose, and it showed apparent decreases in all the time groups in the case of the 9.6 mg/kg dose. It was reported that the Ca^{2+} and Ca levels significantly decrease 60 min

after the intravenous administration of HFA (9.6 mg/kg) in rats⁹). Hypocalcemia in response to F exposure has been explained by the formation of insoluble CaF_2 , which is inaccessible to the intravascular pool¹⁴). Although the serum concentration of F rapidly decreased, the Ca and Ca^{2+} levels did not recover until 300 min. Furthermore, it has been proposed that the increases parathyroid hormone (PTH) levels occur primarily as a consequence of hypocalcemia¹⁶). Therefore, it is possible that there are other mechanisms underlying the decrease in the serum Ca and Ca^{2+} levels. Simpson et al. postulated that formation of fluorapatite rather than formation of CaF_2 is responsible for the decrease of the plasma calcium concentration¹⁷). As a mechanism of fluorapatite formation caused by F, two *in vitro* studies showed that hydroxyapatite acts as a nucleation catalyst for fluorapatite formation and that collagenous matrix induced a similar fluorapatite formation^{18,19}). Because rapid formation of fluorapatite occurred after the addition of a trace amount of F irrespective of the presence of hydroxyapatite in the circulation¹⁸), this process would have been responsible for the hypocalcemia induced by the low serum concentration of F. The amount of $\text{Ca} \times \text{P}$ product decreased in the 5-, 10-, and 30-min groups and the P level increased in the 120- and 300-min groups in the case of the 9.6 mg/kg dose. The P level secondarily increased to compensate for the decrease in the amount of $\text{Ca} \times \text{P}$ product. The pH was decreased only in the 5-min group in the case of the 9.6 mg/kg dose. It was reported that pH kept within the reference range 60 min after the intravenous administration of HFA (9.6 mg/kg) in the rat⁹). Although pH and BE decreased, there were no significant differences of BUN, Cr, P, K, PO_2 and PCO_2 after 5 min compared with the control. The decrease in pH and BE would be the immediate reaction to HFA administration. PO_2 increased

and PCO_2 decreased in the 30-, 60- and 120-min groups in the case of the 9.6 mg/kg dose. It was reported that metabolic acidosis primarily occurs 60 min after the intravenous administration of HFA at concentrations greater than 6.4 mg/kg in rats, and the PCO_2 , HCO_3^- level and BE decrease, whereas the PO_2 increases, because of consequent respiratory compensation⁹. Therefore, we consider that the changes in PO_2 and PCO_2 at 30 to 120 min are attributable to respiratory compensation for metabolic acidosis. On the other hand, the BE and HCO_3^- levels did not recover from 30 to 300 min in the case of the 9.6 mg/kg dose. As a mechanism of metabolic acidosis caused by F, it was reported that the HCO_3^- level and BE decreased owing to renal tubular acidosis 60 min after HFA administration (9.6 mg/kg)⁹. It was also postulated that formation of fluorapatite requires dissociation of HPO_4^{2-} , which releases protons causing metabolic acidosis¹⁸. These combined effects would delay the recovery of BE and HCO_3^- levels. In the 1.6 and 9.6 mg/kg groups, the mean F concentration rapidly decreased, and no apparent differences were found in the 0- and 300-min groups. AUC increased in proportion to the dose, which indicated that there were no apparent retentions of HFA in serum. It was previously found that F clearance is dependent on renal function²⁰. The kidney is the main target of F toxicity. We propose that the elimination of HFA is decreased by the deterioration of renal function; however, it has also been proposed that the serum concentration of F is decreased by the short half-life of F^{21} , the formation of CaF_2 ¹⁴ and fluorapatite¹⁸. Therefore AUC and the serum concentration of F in the 300-min group did not increase considerably. We consider that the harmful effects of HFA are strongly correlated with C_{max} because of the short $\text{T}_{1/2}$.

Conclusions

The serum electrolyte abnormalities were rapidly caused and lasted for several hours, even at the sublethal dose. Furthermore, metabolic acidosis persisted and renal dysfunction was aggravated after elimination of HFA from blood. Therefore, persons involved in HFA accidental exposure should be closely monitored over time.

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