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Brief Report

Toxicokinetics and Metabolism Deteriorated by Acute Nephrotoxicity after a Single Intravenous Injection of Hydrofluoric Acid in Rats

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Abstract: Toxicokinetics and Metabolism Deteriorated by Acute Nephrotoxicity after a Single Intravenous Injection of Hydrofluoric Acid in Rats: Go MITSUI, *et al*. Department of Hygiene and Public Health I · II, Osaka Medical College—Objectives: This study was designed to investigate the early dynamic state of hydrofluoric acid (HFA) in blood and urine as a model of accidental occupational exposure to a subtoxic dose of HFA. It was also aimed at determining the relationship between the kinetics and harmful effects of HFA on the kidney. **Methods**: Rats received a single intravenous injection of HFA (3.2, 6.4, or 9.6 (LD_5) mg/kg) or saline. The volume of each injection was 1 ml and the concentrations of HFA were 0.1, 0.2, and 0.3%, respectively. Ionized fluoride (F) was measured for the biological monitoring of HFA. Serum F concentrations were determined at 0, 5, 10, 30, 60, 120, and 300 min. Pharmacokinetic parameters were calculated with two-compartment modeling. Urine was directly collected from bladder for 300 min to determine the extent of the renal damage. **Results**: $AUC_{0\rightarrow300}$ values were significantly higher in the 9.6 mg/kg group than in the 3.2 and 6.4 groups. The total body clearance, V_1, V_2 and V_{ss} were significantly lower in the 6.4 and 9.6 mg/kg groups than in the 3.2 mg/kg group. These results indicate that HFA was retained in blood. This could be a result of renal dysfunction. NAG/Cr and glucose excretion amount in urine were increased, and the clearance rate of F, urine volume and excretion amounts of electrolytes were decreased in the 9.6 mg/kg group compared with the saline group. These findings indicate renal tubular damage and a decrease in the amount of excretion of HFA from the kidney. **Conclusions**: We consider that acute nephrotoxicity of HFA caused renal injury, and the harmful effects of HFA were subsequently aggravated by its delayed metabolism.
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Key words: Acute exposure, Hydrofluoric acid, Kinetics, Metabolism, Renal injury

Hydrofluoric acid (HFA) is an aqueous solution of liquefied hydrogen fluoride. It is colorless, aquaphilic and highly corrosive. HFA (5–70%) has many uses in various industrial fields, including as a raw material for fluoric chemical products, an acid detergent and for washing semiconductors. It is usually employed in the liquid state at room temperature, because its boiling point is 104°C. As soon as HFA solution accidentally splashes onto the skin of workers, severe chemical burns occur and HFA rapidly infiltrates the systemic circulation through subcutaneous tissues. Generally, exposure to highly concentrated HFA results in a high rate of mortality after several hours[1]. Many clinical cases have shown that abnormal levels of serum electrolytes are strongly connected to mortality[2–6]. We previously reported an acute lethal case within 1 h after exposure to HFA solution, even though it was diluted to a concentration below 5%, which is the minimum for industrial use[2]. It was supposed that the lethal effects of HFA were promoted by exposure conditions that cause rapid absorption of HFA into the body. In our previous study, the acute toxicity of HFA after intravenous infusion to rats was investigated as accidental inhalation or skin exposure. The 24-hour median lethal dose (LD_{50}) was 17.4 mg/kg. Harmful systemic effects were also observed 1 h after exposure to a sublethal dose. The maximum dose was determined as 9.6 mg/kg (LD_5). Rats were injected with HFA (3.2, 6.4, or 9.6 mg/kg) or saline. Acute renal dysfunction and electrolyte abnormalities were observed to be significant and dose-dependent[3]. These

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results showed that even low-concentration exposure to HFA can cause harmful systemic effects in the early stage after acute exposure. It has been reported that the kidney is the main target organ of ionized fluoride (F)[4]. However, there are no experimental reports about damage to glomeruli and tubules of the kidney after exposure to HFA. Furthermore, it has been suggested that HFA retention in blood aggravates other serum disorders, because the kidney is also part of the main pathway of F in the body[5]. However, the degree to which the nephrotoxicity and metabolic kinetics of HFA are affected by renal dysfunction was unclear. Therefore, this study was designed to investigate the early state of HFA dynamics in the blood and urine as a model of acute occupational exposure to a subtoxic dose of HFA. The aims of this study were to determine the extent to which the kinetics and metabolism of HFA vary with dose and to investigate the relationships between F kinetics and the harmful effects of HFA on the kidneys following acute exposure to HFA.

Methods

Nine-week-old specific pathogen free (SPF) male Sprague-Dawley rats weighing 290–300 g were obtained from Japan SLC, Inc. (Shizuoka, Japan). The animals had free access to rat chow (Funabashi Farm MM-3; Chiba, Japan) and tap water, and were housed in separate rooms at a constant temperature (22.0 ± 1.0°C) under a 12-hour light/dark cycle. All aspects of this study were conducted under the guidelines of the Osaka Medical College Ethical Association for Accreditation of Laboratory Animal Care. HFA (concentration, 46%) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All the chemicals were dissolved in or diluted with saline.

Twenty rats were divided into four exposure groups (n=5 for each group). All the rats were anesthetized with sodium pentobarbital. HFA and saline were injected through the tail vein using a syringe pump (model PHD 200P; Harvard Apparatus, Inc., Holliston, MA, USA) for 1 min. Doses of 3.2, 6.4, or 9.6 mg/kg were selected for this study; the ratio of the doses was 1:2:3, and the HFA concentrations were 0.1, 0.2, and 0.3%, respectively. The dose 9.6 mg/kg corresponds to LD_5 and is the highest recorded sublethal concentration at 24 h after exposure. Therefore, it was selected as the maximum dose. Saline was administered to the control group.

Repeated blood sampling

Under sodium pentobarbital anesthesia, the carotid artery was catheterized (24-G indwelling needle; Terumo Corp., Tokyo, Japan), to obtain blood samples (0.4 m*l*). Serum F concentration was determined by the F-selective electrode method (Orion Model 720 AQ) 0, 5, 10, 30, 60, 120, and 300 min after HFA injection. The serum concentration-time profiles of F measured after i.v. HFA injection were analyzed by compartmental modeling with a nonlinear least-squares regression program (WinNonlin; Pharsight Corp., Cary, NC). The WinNonlin program calculates model predictions using standard equations[7]. The best fit of the data was obtained with a two-compartment model rather than a one- or three-compartment model, with a $1/(\hat{Y})^2$ iterative weighting scheme, where $\hat{Y}$ is the serum F concentration predicted by the model. Two-compartment parameter estimates from the regression analysis included the half-life of F in the α phase ($T_{1/2\alpha}$), the half-life of F in the β phase ($T_{1/2\beta}$), the total body clearance (Cl), the apparent volume of distribution at the steady state (V_{ss}), the rate constant for F transfer from the central to the peripheral compartment (K_{12}), the rate constant for F return from the peripheral to the central compartment (K_{21}), the elimination rate constant from the central compartment (K_{10}), and the mean residence time (MRT). The apparent volume of the peripheral compartment (V_2) was calculated as $V_2=V_1 \cdot K_{12}/K_{21}$, where V_1 is the volume of the central compartment. A noncompartmental modeling method was applied to determine the area under the curve values ($AUC_{0\rightarrow300}$).

Urine sampling

Twenty rats were divided into four groups (n = 5 for each group). All the rats were anesthetized with sodium pentobarbital. HFA (3.2, 6.4, or 9.6 mg/kg) or saline was injected as described above. After the injection, saline was administered for 30 min (0.6 m*l*/min) to ensure an adequate urine volume. Urine was accumulated for 300 min after the injection and was obtained with an indwelling catheter (18-G indwelling needle; Terumo Corp.). Catheters were inserted at a 20° angle. The following urinary variables were measured: urine volume, amounts of F, sodium (Na), potassium (K), calcium (Ca), phosphate (P), *N*-acetyl-β-d-glucosaminidase (NAG), creatinine (Cr), NAG/Cr (NAG Cr ratio: the amount of NAG was corrected by the Cr amount because it appeared to be affected by urine volume) and glucose. Total activities and weights were calculated, taking into consideration the variations in urine volume. The F clearance rate was calculated.

Measurement of total F excretion amount

Serum and urinary F concentrations were determined by the F-selective electrode method. The total F excretion amount was calculated taking into consideration differences in urine volume.

Statistical analysis

Data are expressed as mean ± SD. Statistical analysis was performed with SPSS software (SPSS Inc., Chicago, IL, USA). $p<0.05$ was considered statistically significant.

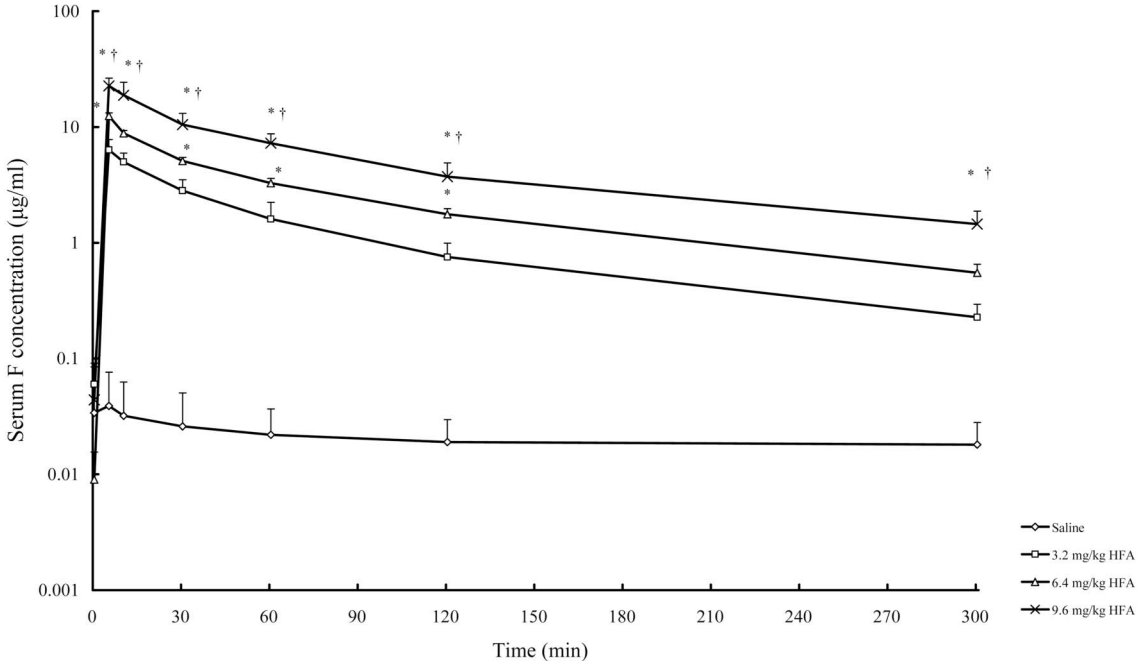

Fig. 1. Ionized fluoride concentraions in serum at 5, 10, 30, 60, 120 and 300 min after a single intravenous injection of hydrofluoric acid (HFA) or saline. Mean ± SD; n = 5; *p<0.05 vs. 3.2 mg/kg, †p<0.05 vs. 6.4 mg/kg by Fisher's protected LSD. Simple regeression analysis was carried out to assess the relationship between F concentration and time. The parallelism of these regressive lines was tested by analysis of covariance. Changes in the time course differed siginificantly among the three groups.

Overall differences among the groups were evaluated by one-way ANOVA. When the results of one-way ANOVA were significant, the differences between individual groups were evaluated by Fisher's protected least significant difference (LSD) test.

Results

The time courses of serum F concentration are shown in Fig. 1. Serum F concentration rapidly increased in all the groups in the first 5 min. The mean serum F concentrations in the 9.6 mg/kg group were significantly higher than those in the 3.2 and 6.4 mg/kg groups at all times. The mean serum F concentrations in all HFA-treated groups were significantly higher than that of the control group at 300 min.

The kinetic parameters are shown in Table 1. $T_{1/2\alpha}$ did not differ among the three groups. The mean of $T_{1/2\beta}$ of the 9.6 mg/kg group increased significantly compared with those of the 3.2 and 6.4 mg/kg groups. The mean of Cl of the 9.6 mg/kg group decreased significantly compared with those of the 3.2 and 6.4 mg/kg groups. The mean of V_1 of the 9.6 mg/kg group decreased significantly compared with those of the 3.2 and 6.4 mg/kg groups. The means of V_2 and V_{ss} significantly decreased dose-dependently in the 6.4 and 9.6 mg/kg groups compared with those of the 3.2 mg/kg group. The means of K_{12} and K_{21} showed no marked changes. The mean of K_{10} decreased dose-dependently and significantly in the 9.6 mg/kg group compared with that of the 3.2 mg/kg group. The mean of MRT increased dose-dependently and significantly in the 9.6 mg/kg group compared with those of the 3.2 and 6.4 mg/kg groups. The means of the $AUC_{0\rightarrow300}$ values of the 6.4 and 9.6 mg/kg groups were significantly higher than those of the 3.2 mg/kg group. The mean $AUC_{0\rightarrow300}$ values of the 6.4 mg/kg group were about twofold higher than those of the 3.2 mg/kg group. The means of $AUC_{0\rightarrow300}$ values of the 9.6 mg/kg group increased beyond the dosage ratio.

The urine volumes and urinary excretion amounts for 300 min after injection are shown in Table 2. The mean urine volume increased significantly in the 3.2 mg/kg group compared with that in the saline group. It tended to decrease in the 6.4 mg/kg group and it was significantly decreased in the 9.6 mg/kg group compared with that in the saline group. The mean urinary F excretion amounts in the 6.4 and 9.6 mg/kg groups increased significantly compared with that of the 3.2 mg/kg group, but that in the 9.6 mg/kg group did not differ from that in the 6.4 mg/kg group. The mean urinary Na excretion amount increased significantly in the 3.2 mg/kg group compared

Table 1. Kinetic parameters calculated from serum F concentrations at 5, 10, 30, 60, 120, and 300 min after a single intravenous injection of HFA (3.2, 6.4 or 9.6 mg/kg)

Variables	HFA (mg/kg)		
	3.2	6.4	9.6
$T_{1/2\alpha}$ (min)	16.3 ± 2.6	11.7 ± 3.3	15.8 ± 5.2
$T_{1/2\beta}$ (min)	108.9 ± 8.6	104.3 ± 15.5	137.6 ± 27.1*†
Cl (m*l*/min/kg)	2.98 ± 0.72	1.36 ± 0.08*	0.63 ± 0.16*†
V_1 (m*l*/kg)	152.7 ± 31.0	74.4 ± 7.6*	42.7 ± 10.5*†
V_2 (m*l*/kg)	172 ± 64	89.1 ± 14.0*	52.8 ± 12.6*
V_{SS} (m*l*/kg)	324 ± 0.084	163.5 ± 16.0*	95.5 ± 22.0*
K_{12} (10^{-2}/min)	1.57 ± 0.39	2.77 ± 0.1	2.19 ± 0.13
K_{21} (10^{-2}/min)	1.45 ± 0.33	2.31 ± 0.68	1.79 ± 1.02
K_{10} (10^{-2}/min)	1.96 ± 0.35	1.85 ± 0.28	1.49 ± 0.14*
MRT (min)	108.6 ± 11.1	120.3 ± 14.8	151.9 ± 17.7*†
$AUC_{0\rightarrow300}$ (μg·min/m*l*)	368 ± 98	752 ± 38*	1365 ± 439*†

A two-compartment model was used with $1/(\hat{Y})^2$ weighting, where $\hat{Y}$ is the serum F concentration predicted using the model. AUC was calculated using a noncompartmental modeling method. Mean ± SD; n=5; *p<0.05 vs. 3.2 mg/kg, †p<0.05 vs. 6.4 mg/kg by Fisher's protected LSD.

Table 2. Urine volumes and urinary excretion amounts during 300 min of urine collection after a single intravenous injection of saline or HFA (3.2, 6.4, or 9.6 mg/kg)

Values	Saline	HFA (mg/kg)		
		3.2	6.4	9.6
Urine volume (m*l*)	6.1 ± 2.1	9.7 ± 2.7*	4.1 ± 2.2	2.6 ± 0.7*
F (μg)	6.0 ± 0.9	134 ± 9*	211 ± 119*	253 ± 106*†(NS)
Na (mEq)	0.83 ± 0.2	1.23 ± 0.45*	0.45 ± 0.3	0.2 ± 0.1*
K (mEq)	0.38 ± 0.1	0.32 ± 0.1	0.18 ± 0.1*	0.18 ± 0.1*
Ca (mEq)	0.13 ± 0.02	0.16 ± 0.05	0.07 ± 0.03*	0.04 ± 0.02*
P (mEq)	9.3 ± 2.2	6.5 ± 3.2*	3.3 ± 1.7*	3.8 ± 1.2*
NAG/Cr (U/g)	38 ± 9	57 ± 39	66 ± 26	121 ± 30*
Glucose (mg)	0.38 ± 0.03	1.35 ± 0.26	1.49 ± 0.9	7.1 ± 8.5*
CF (m*l*/min)	0.89 ± 0.40	2.06 ± 1.01	1.30 ± 0.82	0.6 ± 0.43†

Mean ± SD; n=5; Fisher's protected LSD; *p<0.05 vs. saline, †p<0.05 vs. 3.2 mg/kg of HFA. NS: not significant vs. 6.4 mg/kg of HFA, NAG/Cr: NAG Cr ratio, CF: clearance rate of ionized fluoride. Total volume and excretion amounts were calculated taking variation of urine volume into consideration.

with that in the saline group. That in the 6.4 mg/kg group tended to decrease. It decreased significantly in the 9.6 mg/kg group compared with that in the saline group. The mean urinary excretion amounts of K and Ca decreased significantly in the 6.4 and 9.6 mg/kg groups compared with that in the saline group. The mean P excretion amounts decreased significantly in all the HFA treated groups compared with that in the saline group. The mean NAG/Cr and glucose excretion amount increased in a dose-dependent manner in the 6.4 and 9.6 mg/kg groups and significantly increased in the 9.6 mg/kg group compared with that in the saline group. The mean clearance rate of F decreased significantly in the 9.6 mg/kg group compared with that in the 3.2 mg/kg group.

Discussion

It has been reported that BUN and Cr amounts increase 24 h after inhalation of HFA or severe cutaneous exposure in humans[1, 6]. Dote reported on the nephrotoxicity of F and revealed that an infusion of sodium F solution caused acute glomerular dysfunction and proximal renal tubular injury within 6 h in rats[4]. In our previous study, blood samples were obtained from the carotid artery 1 h after the infusion of HFA (3.2, 6.4 and 9.6 mg/kg). BUN and Cr levels significantly increased in the 3.2, 6.4, and 9.6 mg/kg groups compared with in the saline group. It was

considered that the significant increases in BUN and Cr amounts observed in the previous study were due to acute renal failure.

In this study, the mean urine volume tended to decrease in the 6.4 mg/kg group and it significantly decreased in the 9.6 mg/kg group compared with that in the saline group. Furthermore, the mean serum F concentrations in the 9.6 mg/kg group were significantly higher than those in the 3.2 and 6.4 mg/kg groups at all times. The means of $T_{1/2\beta}$ of the 9.6 mg/kg group increased significantly compared with those of the 3.2 and 6.4 mg/kg groups. The mean of Cl of the 9.6 mg/kg group decreased significantly compared with those of the 3.2 and 6.4 mg/kg groups. It is reported that F clearance is dependent on the glomerular filtration rate because glomerular filtration is the major pathway of F elimination from the human body, and there is a direct correlation of F clearance with urinary flow[5]. Therefore, we considered that a higher dose would cause a more severe renal dysfunction in this study.

It was reported that urine volume increased after a single intravenous injection of 3 mg/kg F in rats, and it decreased after administration of 6 or 9 mg/kg F[4]. Oral administration of F also caused polyuria[7]. These results suggest that the increased urine volume in the 3.2 mg/kg group in this study was caused by the diuretic action of F, despite the occurrence of renal dysfunction. Urinary F analysis has been recognized as suitable for the biological monitoring of occupational HFA exposure, because F is mainly excreted via the kidney[8]. However, the mean urinary F excretion amount in the 9.6 mg/kg group did not differ from that in the 6.4 mg/kg group. It was reported that once renal function has diminished to an extremely low level, the excretion amount of F in the urine decreases and serum F concentration increase[5]. Therefore, we consider that severe renal dysfunction in the 9.6 mg/kg group decreased urinary F excretion and the clearance rate of ionized fluoride (CF). We also consider that renal tubular injury decreased the urinary excretion of K, and increased NAG/Cr and glucose excretion in the 6.4 and the 9.6 mg/kg groups. Our previous study showed that the serum K level of the 9.6 mg/kg group significantly increased compared with that of the control, and that metabolic acidosis was complicated[3]. Therefore, we consider that renal dysfunction mainly decreased the amount of urinary excretion of HFA from the blood. Furthermore, retention of HFA would aggravate renal injury and abnormalities in levels of serum electrolytes.

Conclusions

Even if HFA exposure occurs at a subtoxic dose below the minimum for industrial use, acute renal dysfunction would be caused by its nephrotoxicity, and the systemic harmful effects would be subsequently aggravated by disorders of the kinetics and metabolism of HFA. Thus, it is necessary for individuals working with even highly-diluted solutions of HFA to take extreme precautions.

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