Original

Lethal Effects on Blood and Lung during Acute Respiratory Distress after Inhalation Exposure of Rats to Aerosolized Hydrofluoric Acid

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Abstract

The purpose of this study was to reveal the mechanism of acute lethal accident after the exposure of human on face to a diluted solution of hydrofluoric acid (HFA). Particles of HFA solution and saline (50μL) were intratracheally (i.t.) sprayed to rats using an aerosol generator as examples of inhalation exposure to HFA and control, respectively. A lethal dose (LD) study showed that the 24h $LD_{50\ i.t.}$ and $LD_{99i.t.}$ were 0.58 and 1.92mg/kg, respectively; 1.92mg/kg (1.2%) can cause mortality within several hours after the exposure to satisfy the model of the accident. The blood, bronchoalveolar lavage fluid (BALF), and lung tissues were sampled 1h after the i.t. administration of HFA (1.92mg/kg) and saline for the biological exposure and effect monitoring. Blood parameters indicated severe respiratory acidosis without apparent abnormalities of serum electrolytes. Semiquantitative grading of pathohistological findings revealed that there were mild hemorrhage and effusion in alveolar spaces in the HFA group. The level of surfactant protein D in the BALF of the HFA group was much lower than that of the control. It was considered that the respiratory failure would be mainly due to the deficiency of the surface acting agent in the alveolus caused by the retained HFA in the alveolar spaces. Hyperpotacemia and hypocalcemia were the main causes of mortalities in many clinical cases after HFA exposure. However, it was confirmed that the strong mortality after the inhalation of HFA aerosols could be derived from the pulmonary dysfunction without abnormalities of serum electrolytes in this study.

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—Key words—

hydrofluoric acid, inhalation, respiratory failure, bronchoalveolar lavage fluid, surfactant protein D, lethal mechanism

Introduction

Gaseous hydrogen fluoride (HF) has been used to investigate the harmful effects of inhalation exposure in many reports[1]. Hydrofluoric acid (HFA) is a watery solution of liquefied HF, which has a marked reactive property. HFA is the convenient form for the preservation and use of HF, because the boiling points of liquid HF and HFA are 19 and 104℃ (azeotropic tempareture), respectively. Because a high concentration of HFA can rapidly activate chemical reactions, HFA (5–70%) is commonly used in a variety of chemical industries such as etching semiconductor devices and petrochemical manufacturing. On the other hand, there were many case reports concerning poor prognosis after accidental exposure. Typical clinical courses have been considered as follows. Dermal exposure to a high concentration of HFA was followed by a rapid and efficient absorption into the blood through the skin. It resulted in serum chemical disorders such as metabolic acidosis and abnormalities of electrolytes that mainly cause cardiac dysfunction several hours after the accident[2)~4)]. Our previous clinical case showed that a 65-year-old man subsequently succumbed to respiratory failure within 1 hour after the occupational exposure of HFA on his face. Autopsy revealed the following findings. Bilateral lungs were severely congested and edematous in the macroscopic findings. Histopathological findings showed alveolar tissues with eosinophilic substances within the alveoli and no severe destruction of interstitial tissues. Therefore, it was considered that the cause of death would be a combination of pulmonary disorders with cardiac dysfunc-

tion that might be caused by hyperpotacemia and hypocalcemia. Although the HFA solution was highly diluted (<5%) and the exposed surface area was narrow, the clinical course was rapid deterioration to death[5]. Therefore, it was considered that the main lethal route would be inhalation of HFA rather than dermal exposure. Then, it was hypothesized that inhalation exposure causes high mortality, even if the concentration is below 5%, which is the minimum for industrial uses. In many industrial fields, it was considered that particles of HFA solution are probably sprayed into the air under some operations, such as spraying, agitation and transfer. It will possibly be risky that workers inhale massively the floating mist of HFA during breathing. Therefore, the useful planning of emergency procedures to reveal the eventual response to aerosolized HFA is necessary. Nevertheless, there are no research studies on the acute inhalation exposure of aerosolized HFA. The lethal mechanism is also unknown, whether respiratory dysfunction caused by lung injury or harmful systemic effects after absorption into the blood. Therefore, this study confirmed the toxicity and systemic effects after inhalation of aerosolized HFA. Furthermore, the mechanism of mortality was also explored with emphasis on acute pulmonary injury.

Materials and Methods

Animals

Ten-week-old specific-pathogen-free male Sprague-Dawley rats weighing 290–300g 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±1.0℃) under a 12-hour light/dark cycle. Rats were given only tap water for 18 hours before experiments to defuse the effect of F contained in the diets. All aspects of these studies were conducted following the guidelines of the Osaka Medical Ethical Association for Accreditation of Laboratory Animal Care.

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

Model of inhalation exposure of HFA

In many accidental exposures, the HFA solution would be converted into aerosol by rapid flushing. The particles were extensively inhaled into peripheral airways. It was hypothesized that the lethal effect was caused by massive expansion of HFA over respiratory tracts. Therefore, the aerosolization was applied to the experimental model of this lethal case.

Generation of HFA aerosol

Aerosolized particles of 16–22μm MMD (mean drop diameter) were sprayed using the aerosol generator, which consists of a tubing (Sprayer™A-1C, Penn-Century, Inc.) and a high-pressure syringe (FMJ-250, Penn-Century, Inc.). Fifty microliters of solution at room temperature was administered into the lungs.

Intratracheal (i.t.) administration of HFA

All the rats were anesthetized by inhalation of isoflurane (ISOFLU®, Dainippon Sumitomo Pharma Co., Ltd.) using the inhalation anesthesia apparatus (Univentor 400 Anesthesia Unit, Univentor Ltd.). Previous determination in rats of the same size had indicated that, at this distance of insertion, the tip of the tubing was located 1–2mm above the bifurcation on the trachea. For the administration, the tubing was inserted by direct laryngoscopic orotracheal intubation using the small-animal laryngoscope (LS-1, Penn-Century, Inc.); precise placement of the tubing was facilitated by reference marking on the tubing. With the tubing thus positioned, the solution was sprayed, and the tubing was then withdrawn completely.

Lethal dose study

Forty-two rats were assigned to one of seven exposure groups ($n=6$ in each group). One of seven concentrations of HFA solution (0.025, 0.05, 0.1, 0.2, 0.4, 0.8 or 1.2%) was administrated. Doses of the HFA were 0.04, 0.08, 0.16, 0.32, 0.64, 1.28 and 1.92mg/kg, respectively. A probit dose-mortality curve was generated based on mortality after 24 hours[6], and the lethal dose of HFA was determined using SPSS® software (Chicago, IL).

Acute exposure to the toxic dose

Twelve rats were assigned to one of two exposure groups ($n = 5$ for biochemical study, $n = 1$ for pathological study in each group).

In accordance with the dose-mortality curve, the dose of the HFA-treated group was set as 1.92mg/kg (1.2%, 50μL). This dose corresponded to LD_{99} and it satisfies the requirement that the lethal effects should be observed within several hours after the exposure. Saline was used as a control.

Blood sampling

Blood samples were collected from the carotid artery 1 hour after the administration.

Biological effect monitoring

Serum parameters

Our previous study showed that kidney dysfunction, acute electrolyte disturbance, and metabolic acidosis occurred 1 hour after the intravenous administration of HFA[7]. Therefore, the following parameters were measured according to standard protocols recommended by assay suppliers: blood urea nitrogen (BUN, urease-glutamic dehydrogenase method), creatinine (Cr, Jaffe method), sodium (Na, Na^+-selective electrode method), potassium (K, K^+-selective electrode method), chlorine (Cl, Cl^--selective electrode method), total calcium (Ca, OCPC method), and phosphate (P, molybdic acid UV method).

Arterial blood gas analysis

The pH, PO_2 (mmHg), PCO_2 (mmHg), HCO_3^- (mmol/L), and base excess (BE) (mmol/L) (288 Blood Gas System; Bayel, Osaka, Japan) were determined.

Biological exposure monitoring of HFA

The serum concentration of HFA was investigated on the basis of ionized fluoride (F, F-selective electrode method).

Bronchoalveolar lavage fluid (BALF) sampling

Rats were sacrificed by exsanguinations after the blood samplings. Pneumothorax was produced by cutting the diaphragm, and the chest cavity was opened. The trachea was exposed, and the anterior half was cut. A tube was inserted through the tracheal hole. PBS (5mL) was infused into the trachea (10ml/min) and vacuumed out (1ml/min) twice using the infusion pump (model PHD 200P; Harvard Apparatus, Holliston, MA). The total lavage volume was 10 mL. Samples were stored in 15-mL polypropylene conical tubes (Falcon®; BD Biosciences, Franklin Lakes, NJ). First, the recovery rates of BALF were calculated. Second, the samples were centrifuged for 10 min at 1,000 rpm at 4℃ and stored on ice until cell count analysis.

Chemical and cellular measurements of BALF

Surfactant protein D (SP-D) (E-CLISA); LDH (UV method)

SP-D, a type of phosphorus lipid-protein complex, is produced by type II pulmonary alveolus, which is prevented from collapsing by being secreted in the alveolar space. Serum SP-D is clinically a useful biomarker for evaluating lung diseases without the need for biopsy or lavage[8]. Because SP-D in serum would possibly arise in part from extrapulmonary sites, SP-D in BALF reflects the events occurring in the involved lung more directly[9]. Therefore, it was evaluated as a biomarker of several injury models as well as LDH in rat[10].

F concentrations in BALF

The concentrations of BALF were also measured to investigate the retention of HFA in the lungs.

Total cell counts in BALF

Supernatants were removed and 1mL of PBS was added and mixed. Cell samples were mixed with trypan blue solution 10 times, and separate counts were made from each tube; separate samples were placed in the top and bottom chambers of an AO Spencer Bright-Line hemocytometer (St. Louis, MO) and analyzed by light microscopy (×400). The total was the number of cells in all the samples.

Cell analysis in BALF

Each cell solution (20μL) was dropped onto a slide glass and dried. Cells were fixed in ethanol and Diff-Quick. One hundred cells were counted randomly and classified in terms of shape, size and color.

Sampling of lung tissues

Tubes were inserted into the trachea. Collapsed lungs were then infused with 10% neutral-buffered for-

Table 1 Serum parameters 1 h after intratracheal administration of saline or HFA (1.92mg/kg).

		Saline	HFA (1.92 mg/kg)
Na	(mEq/l)	143.4 ± 2.3	141.0 ± 9.2
K	(mEq/l)	4.5 ± 0.5	4.5 ± 1.4
Cl	(mEq/l)	105 ± 3	100 ± 7
Ca	(mg/dl)	10.4 ± 0.2	10.4 ± 0.4
P	(mg/dl)	7.4 ± 0.9	11.7 ± 2.8 *
BUN	(mg/dl)	17.1 ± 2.2	22 ± 1.9 *
Cr	(mg/dl)	0.27 ± 0.05	0.25 ± 0.09
Fluoride	(μg/L)	3.8 ± 0.9	630 ± 210 *

Mean ± SD, n = 5 Student's t-test; *$P < 0.05$ vs. saline

Table 2 Blood gas analyses 1 h after intratracheal administration of saline or HFA (1.92 mg/kg).

		Saline	HFA (1.92 mg/kg)
pH		7.37 ± 0.05	7.15 ± 0.13*
PCO_2	(mmHg)	44.8 ± 6.6	82.3 ± 26.6*
PO_2	(mmHg)	84.5 ± 15.9	46.2 ± 11.7*
HCO_3^-	(mmol/l)	25.5 ± 1.4	27.1 ± 3.7
BE	(mmol/l)	0 ± 0.4	− 3.9 ± 3.8*

Student's t-test; *$P < 0.05$ vs. saline

malin. Lung tissues were mildly extended for clear pathologic observation and fixed with the formalin solution for 24h. The upper and lower lobes of bilateral lungs were sampled. Tissue sections of 2μm thickness were prepared, stained with hematoxylin and eosin (H & E), and analyzed by light microscopy.

Grading of pathohistological findings

Furthermore, the pathohistological findings were assessed semiquantitatively, such as destruction of alveolar architecture, interstitial and intraalveolar edema, effusion and hemorrhage into the alveolar space, and infiltration of inflammatory cells. The severities were graded 0 to 4 [0, no; 1, low number per viewed field; 2, high number per viewed field; 3, many but not all; 4, in all fields], which means no, minimal, mild, moderate and severe changes, respectively

Statistical analysis

Data are expressed as mean ± SD. Differences between the control and treatment groups were analyzed by t-test. Statistical analysis was performed using SPSS® 10.0J software (SPSS Inc., Chicago, IL). Results were considered significant at $P<0.05$.

Results

Lethal dose study

HFA concentrations of 0.04, 0.08, 0.16, 0.32, 0.64, 1.28 and 1.92mg/kg resulted in death of 0, 1, 2, 2, 4, 5 and 6 of six rats within 24 hours, respectively. The lethal dose lowest (LDLo) was 0.08mg/kg. The 24h $LD_{50\ i.t.}$ and $LD_{99i.t.}$ were 0.58 and 1.92mg/kg, respectively. All the rats died within 2h after administration of $LD_{99i.t.}$.

Acute exposure to the toxic dose

General status

Labored breathing and peripheral cyanosis were observed approximately from 0.5h after HFA administration.

Blood findings

Serum biochemical parameters are shown in Table 1. The Na, K, Cl, and Ca levels showed no significant difference between the HFA and control groups and the control group. The means of the P and BUN levels of the HFA group were significantly higher than those of the control. The means of the F levels apparently increased in HFA group.

Arterial blood gas values are shown in Table 2. The means of pH, PO_2, and BE of the HFA group were significantly lower than those of the control group. The means of PCO_2 were about twofold higher than that of the control group. The means of PO_2 of the HFA group were about half of the control. There were no significant differences in the HCO_3^- level between the control and HFA groups. The means of BE of the HFA group were significantly lower than those of the control.

Findings in BALF

The parameters are shown in Table 3. The recovery rate of the HFA group was lower than that of the control. The means of the F level apparently increased in the HFA group. The LDH level of the HFA group was lower than that of the control. The SP-D level in the BALF of the HFA group was about two-fold lower

Table 3 Findings in BALF 1 h after intratracheal administration of saline or HFA (1.92 mg/kg).

		Saline	HFA (1.92 mg/kg)
Recovery rate	(%)	90.8 ± 1.9	86.4 ± 3.3 *
Fluoride	(μg/L)	33 ± 42	138 ± 58 *
LDH	(IU/L)	41.8 ± 5.1	34.8 ± 7.9
SP-D	(ng/ml)	160.2 ± 19.8	61.8 ± 36.4 *
Cell counts	($\times 10^5$/ml)	32.7 ± 8.5	9.4 ± 3.0 *

SP-D: surfactant protein D, Mean ± SD, n = 5 Student's t-test; *$P <$ 0.05 vs. saline

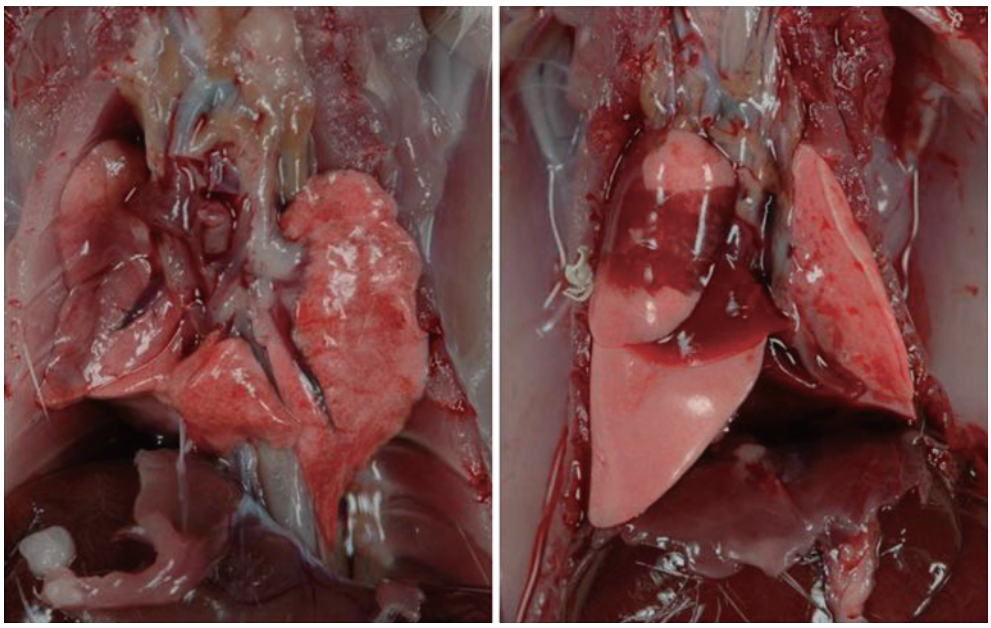

Fig. 1 Macroscopic findings of lungs 1 h after intratracheal administration of saline (left) or HFA (1.92 mg/kg) (right).

than that of the control. The total cell counts of the HFA group were about three-fold lower than that of the control. There were no apparent differences in the cell analysis results between both groups.

Morphological comparison

The macroscopic findings are shown in Fig. 1. In the control group, there were no degeneration, discoloration, and erosion on bilateral pulmonary surfaces (Fig. 1–a). In the HFA group, the right lung showed diffuse edema and intrapulmonary hemorrhage in the upper lobe. The left lung showed slight swelling and scattered reddish spots (Fig. 1–b). The upper lobes of the right lungs were selected for the pathohistological examination, because macroscopic findings showed the most severe damage in these parts in the HFA group.

Fig. 2–a showed the same part of the control group. There were no disorders in the alveolar architectures such as blood vessels, alveoli, alveolar ducts, alveolar septa, terminal bronchioles, and respiratory bronchioles. Bronchiolar epithelial (Ep) cells were also normally arranged. In the alveolar spaces, macrophages were slightly scattered without effusion and hemorrhage. Fig. 2–b, c, d showed those of the HFA group. The HFA group showed no apparent changes in the macrophage, interstitial, intraalveolar, and perivascular regions compared with the control. Moderate eosinophillic proteinaceous fluid effused with moderate hemorrhage in the alveolar spaces (Fig. 2–b). Bronchiolar Ep cells were desquamated. In the alveolar spaces, mild eosinophillic proteinaceous fluid effused with minimal infiltration of macrophages and moderate hemorrhage (Fig. 2–c, d magnification).

Table 4 shows the comparison by grading of pathohistological findings. In the control group, there were no findings in the alveolar architectures such as abnormal arrangements and desquamations of Ep, and no

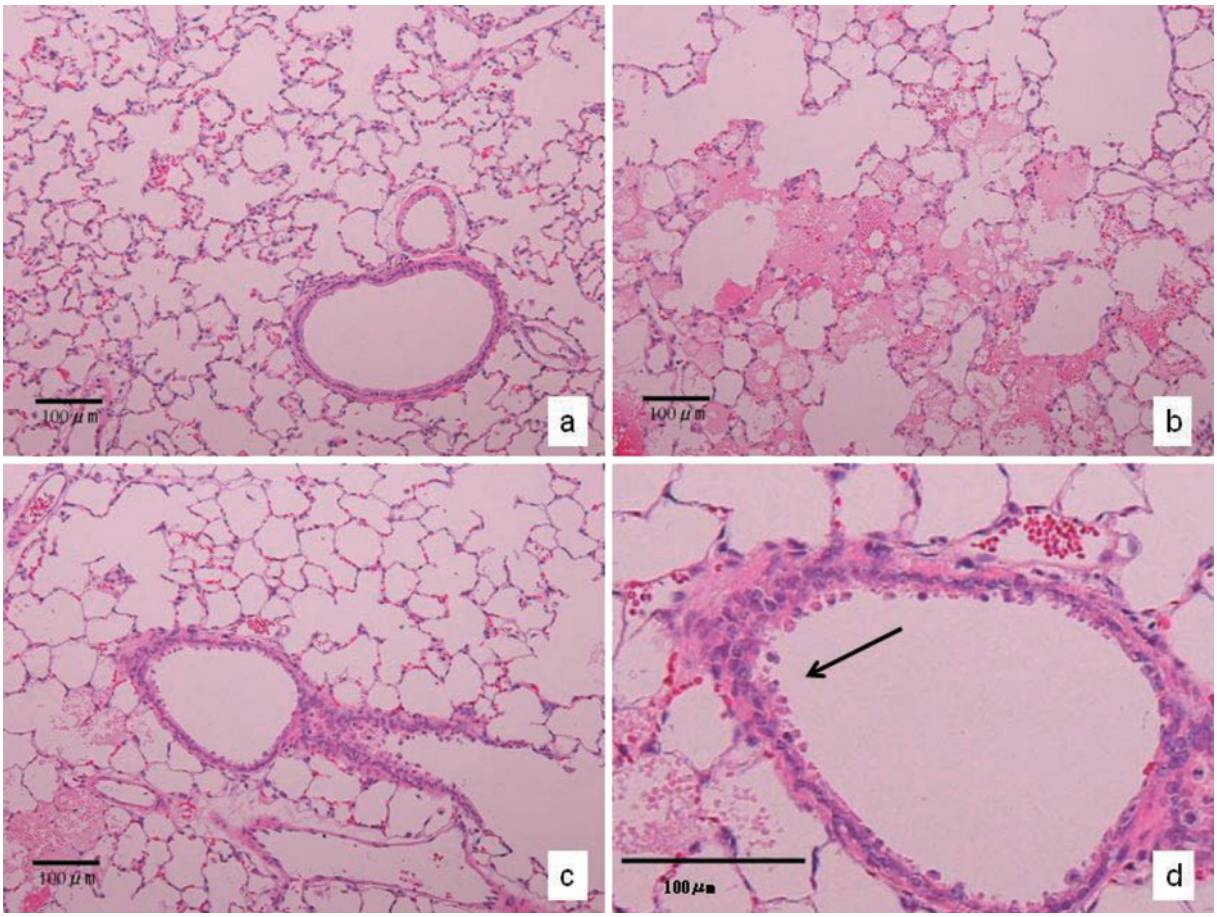

Fig. 2 Pathohistological findings of lungs 1 h after intratracheal administration of saline or HFA (1.92 mg/kg). (H & E stain). a, saline; b, c, eosinophilic proteinaceous fluid effused with hemorrhage; c, d, desquamations of epithelium cell (arrow).

Table 4 Semiquantitative gradings of pathohistological findings in the upper and lower lobes of bilateral lungs.

	Saline				HFA (1.92 mg/kg)			
	Right		Left		Right		Left	
	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Desquamation of bronchiolar epithelium	0	0	0	0	2	1	2	1
Hemorrhage and effusion in alveolar spaces	0	0	0	0	3	3	2	1

The severities were graded 0 to 4 [0, no; 1, low number per viewed field; 2, high number per viewed field; 3, many but not all; 4, in all fields], which means no, minimal, mild, moderate and severe changes, respectively.

hemorrhage and effusion in the alveolar spaces. On the other hand, the abnormal findings and gradings in every part of the lungs were shown in the HFA group. The desquamations of Ep were mild in the upper right, minimal in the lower right, mild in the upper left, and minimal in the lower left lobe. The hemorrhages and effusions were moderate in the upper right, moderate in the lower right, mild in the upper left, and minimal in the lower left lobe.

Discussion

The LDLo by i.t. route was 0.08mg/kg in this study. It was reported that the LDLo values by other exposure routes were 13.1mg/kg by intraveneous (i.v.) route, 25mg/kg by intraperitoneal (i.p.) route, and 100mg/kg by intrasubcutaneous (i.s.c.) route, it was found that the severe harmful systemic effects could be markedly enhanced by inhalation exposure compared with the other routes[7][11]. The Na, K, Cl, and Ca levels showed no significant difference between the HFA and control groups. Therefore, it was suggested that these electrolytes were almost unaffected after the i.t. administration of HFA (1.92mg/kg) in this study. It was suggested that the

P level might be increased by lung injury, because the increased breakdown of cells releases P and may lead to hyperphosphatemia[12]. It was reported that the BUN and Cr levels were increased by renal dysfunction within 24 hours of HFA inhalation in humans[13]. Our previous study also showed that acute glomerular dysfunction caused the increases in the BUN and Cr levels 1 hour after HFA (3.2mg/kg) i.v. administration in rats[7]. Although the BUN level in the HFA group significantly increased, the Cr level did not apparently increase in this study. It was considered that the BUN: Cr ratio was elevated by the increased production of urea such as tissue disruptions and hemolysis[14]. Therefore, it was suggested that HFA (1.92mg/kg) by i.t. route might not cause severe renal dysfunction within 1 hour. It was reported that a high serum concentration of HFA after exposure was the main cause of mortality derived from cardiotoxicity secondary to the hypocalcemia and hypercalcemia in humans[15][16]. Our previous autopsy results showed that the serum F concentration was 63.8μg/ml in the above referenced accident[17]. On the other hand, the mean serum F concentration was 0.63μg/ml 1 hour after the i.t. administration of HFA (1.92mg/kg) in this study. Furthermore, 1.6mg/kg is equal to 1/6 of LD_5 i.v. (9.6mg/kg) in our previous study[7]. Therefore, it was considered that the serum concentration of HFA did not closely correlate with the lethal effect in this study. On the other hand, the respiratory function was not disrupted by i.t. the administration of aerosolized saline. However, pH, PO_2, and BE decreased, and PCO_2 increased significantly in the HFA group. It was considered that they indicated severe respiratory acidosis. It was reported that these changes were also accompanied by the increase in the HCO_3^- level in typical clinical courses. However, there were no significant differences in the HCO_3^- levels between the control and HFA group in this study. It was considered that it had not been compensated by the renal reabsorption of HCO_3^- 1 hour after the exposure, because the renal compensatory mechanisms require more time, generally 6 to 12 hours, to show an effective response[18]. Because blood gas analyses indicated severe respiratory distress as well as the general condition, it was confirmed that the high mortality was derived from the respiratory failure. It was generally considered that there is a negative correlation between lung injury and recovery rate of BALF. In this study, slight decreases in recovery rates in the HFA group might indicate minimal or mild organic destruction of the alveolar architecture. The means of the F level in BALF apparently increased in the HFA group. It indicated that HFA had not yet been excluded from the alveolar spaces 1h after the exposure. Determination of the LDH activity in BALF is useful as a rapid screening test for lung injury in response to exposure to pneumotoxic chemicals[19]. In this study, the LDH level decreased 1h after the HFA exposure. It was suspected that the intraalveolar LDH was sequentially inactivated by the retained HFA. Determination of the total cell counts of BALF is useful for the quantitative evaluation of alveolar injury. During the inflammation of the alveoli and interstitium, the total numbers of all cell types are markedly increased[20]. However, the total cell counts decreased 1h after the HFA exposure in this study. It was reported that the total cell counts decreased 2 hours after the inhalation of gaseous HF in human[21]. Therefore, it was suspected that they were probably destroyed after contact with HFA as well as LDH. Furthermore, the SP-D level in BALF also decreased 1h after the lethal dose of HFA. It was suggested that HFA would directly dissolve the secreted SP-D. It was also hypothesized that the secretion was inhibited by the alveolar cell injury. From these results, it was considered that the respiratory failure would be due to the deficiency of the surface acting agent in the alveolus. On the other hand, it was reported that serum the SP-D levels increased 4h after the intratrachial instillation of hydrochloric acid as a model of moderate lung injury[10]. Therefore, it was considered that the SP-D level in the BALF might be increased by a sublethal dose of HFA after several days. The morphologic changes indicated the extensive abnormalities of the lungs in the HFA group. It was confirmed that the inhaled dust particles may directly sediment in the alveoli if they have a diameter in the range 1–5μm[22]. However, the MMD of HFA was 16–22μm. Therefore, it was considered that the HFA aerosol secondarily diffused to alveolar tissues after the impaction and sedimentation on conducting airways because the HFA easily infiltrates pulmonary tissues owing to its strong corrosive action similar to that of alkali, even if it is diluted. Furthermore, the right lung was damaged more severely as shown from the macroscopic comparison of both sides in the HFA group. It was considered that the swelling was mainly derived from the effusion and hemorrhage into the alveolar spaces rather than interstitial edema. The semiquantitative grading also indicated that the degrees of damage were dominant on the right side. The sectional anatomy of rats indicated that the right main bronchus is more in line

with the trachea and is broader and shorter than the left, similar to that in humans[23]. Therefore, it was considered that the aerosol particles could probably be also anatomically deposited in the right lung rather than the left in clinical cases. It was reported that the acute respiratory distress syndrome was experimentally realized using saline lavage, which depleted the natural lung surfactants, leading to a similar pathophysiological cascade[24]. In this study, the respiratory distress might be mainly caused by the surfactant deficiency, because there was no severe architectural destruction such as liquefactive necroses of the alveolar and interstitial tissues. Moreover moderate hemorrhage and effusion might decrease alveolar diffusing capacity. Therefore, it was considered that the mortality could be derived from respiratory dysfunction without severe pathohistological changes and abnormalities of serum electrolytes. Thus, it was confirmed that the main lethal mechanism after the inhalation of HFA aerosols would be respiratory failure caused by alveolar dysfunction.

Conclusions

Aerosolized HFA could be extensively expanded into the peripheral fields of lungs by invasion activity, even if the concentration is low. Severe respiratory failure could be rapidly derived from the deficiency of surfactant resources in addition to the pulmonary injury. Therefore, the lethal mechanism after inhalation of HFA could be directly caused by the effect on the lungs without harmful abnormalities of serum electrolytes.

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ラットにおけるフッ酸エアロゾル吸入曝露後の血液，肺への致死影響

今西 将史，土手友太郎，河野 公一
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―キーワード―
フッ酸，急性肺障害，吸入曝露

我々はフッ酸（HFA）の吸入曝露後の致死事例を経験し，本誌にて症例報告した．しかし急性毒性の発現がわずか数十分間の経過で重篤な転帰に至る機序は不明であった．また近年，HFA の皮膚曝露事故は後を絶たず，致死機序の仮説があるが，吸入曝露による生体影響や致死機序の報告はない．そこで本研究において吸入曝露後の致死量および急性毒性の発現機序について検討した．曝露形態として溶液粒子の吸入を想定し，エーロゾル発生器を用い，溶液（50μl）を平均粒子径約 20μm にエーロゾル化した．まず経気道的投与後の致死量（intratracheal lethal dose：LD it.）に関してはラットを 7 群に分け，先行研究で得た静注後 24 時間 LD5 iv.の 5 分の 1 を上限とし，7 種類の濃度の HFA（0.04～1.92 mg/kg）を経気道的に単回散布した．24 時間後の死亡数からプロビット法にて推定した 24h LD 50 it.は 0.58mg/kg であった．従って吸入曝露は静脈内投与に比し極めて強い毒性を発現したと考えられた．次に HFA（1.92mg/kg）投与群は 1 時間以内に努力性呼吸が増強し 2 時間以内に全数死亡したため実例に即したモデルと考えられ，急性毒性影響の検討に用いた．ラットを 2 群に分け，HFA 1.92mg/kg およびコントロールとして生理食塩水を同様に投与した．そして 1 時間後の動脈血，肺胞洗浄液（BALF）および肺標本を生化学ならびに病理組織学的に検討した．生体影響に関して HFA 群の血液指標は重篤なガス交換障害および呼吸性アシドーシスを示した．また BALF 中の SP-D, LDH および細胞数の明らかな低下は BALF 中フッ素濃度の上昇から HFA の有害作用に起因したと考えられた．一方，病理組織学的所見において肺胞構造には著明な破壊を認めなかったが，肺胞腔内に滲出液や出血を生じた．従って致死機序として HFA は広範囲に浸透し，滲出液，出血および肺胞の表面活性物質の低減の結果，換気障害を生じたと考えられた．

（日職災医誌，57：109―117，2009）