Original

Process of Early Recovery from Lung Injury Based on Biochemical and Histopathological Findings after Inhalation Exposure of Rats to Aerosolized Hydrofluoric Acid

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Abstract

The purpose of this study was to reveal the lung condition and biological exposure monitoring during the process of recovery after acute inhalation exposure to hydrofluoric acid (HFA) for the modeling of occupational accidents of HFA. Rats were divided into two groups (1 hr. and 48 hrs. groups) according to sampling time. Aerosols of HFA or saline were intratracheally sprayed to the HFA and saline groups, respectively. Blood, bronchoalveolar lavage fluid (BALF), and lung tissues were sampled 1 hr. and 48 hrs. later. Their respiratory states rapidly worsened 1 hr. later, but all the rats survived 48 hrs. after HFA administration. The mean of PO_2 of the HFA (1 hr.) group apparently decreased compared with that of the saline group, but it showed an increasing trend 48 hrs. later. It was assumed that the hypoxemia transitorily appeared 1 hr. later and the respiratory dysfunction tended to improve 48 hrs. later. The SP-D level in BALF was lower in the HFA (1 hr.) group than in the saline group. The SP-D and LDH levels in BALF of the HFA (48 hr.) group were higher than those of the HFA (1 hr.) group. It was assumed that their levels increase for several days after the exposure. The F levels in serum and BALF of the HFA (1 hr.) group showed an increasing trend compared with those of the saline group. There were no significant differences between those of the HFA (48 hr.) group and saline group. It was assumed that HFA was rapidly absorbed from lung tissues to the blood and it was metabolized from the blood 48 hrs. later. The bilateral lungs showed macroscpic swelling and hemorrhage in both HFA groups. The semiquantitative grading of histopathological findings indicated that the prominent findings were the perivascular and alveolar effusion into the alveolar spaces in the HFA (1 hr.) group and thickened bronchial epithelium and desquamations of the bronchial epithelium in the HFA (48 hr.) group. It was suggested that the perivascular and alveolar injury immediately occurred after the early exposure and the thickening of the bronchial epithelium and desquamations of bronchial epithelium occurred a few days later. It was considered that the PO_2 tends to improve in the 48 hrs. with the decrease in the amount of alveolar effusion and the recovery of surfactant protein.

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—Key words—

hydrofluoric acid, inhalation, blood gas, bronchoalveolar lavage fluid, lung injury

Introduction

Hydrofluoric acid (HFA) is an aqueous solution of liquefied hydrogen fluoride (HF). It is colorless, hydrophilic and highly corrosive. HFA (5–70%) has many uses in various industrial fields, including 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℃. 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

closely related to mortality[2)~6)]. 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 would be risky if workers inhale massive amounts of the floating mist of HFA while breathing. In fact, we previously reported that a 65-year old man subsequently died from respiratory failure within 1 hr. after being occupationally exposed to HFA on the face, even though it was diluted to a concentration below 5%, which is the minimum for industrial use[2)]. Autopsy findings showed that bilateral lungs were severely congestive and edematous. The histopathological findings were severe congestion of the capillary vessels and presence of massive eosinophilic substances within the alveoli. Therefore, it was suspected that respiratory dysfunction was of pathogenetic importance in lethal clinical cases. In our previous study, we revealed the mechanism of acute lethal accident 1 hr. after the intratrachial (i.t.) administration at the minimum dose for industrial uses for models of rapid inhalation exposure to HFA[7)]. Respiratory failure could rapidly occur following the deficiency of surfactant resources in addition to pulmonary injury such as alveolar hemorrhage and effusion. It was confirmed that the high mortality after the inhalation of HFA aerosols could be caused by pulmonary dysfunction. Furthermore, another clinical case showed that a 52-year old man inhaled HF while polishing the inside of a stainless steel tank. He was hospitalized with rapid onset of severe dyspnea and the chest radiography results showed diffuses veiling over both lower pulmonary fields. The disorders were improved by the specialized emergency procedures over a few days[8)]. However, there are few useful reports on predicting the early clinical course of acute inhalation exposure to HFA. The useful planning of emergency procedures as an eventual response to aerosolized HFA is necessary. Therefore, the purpose of this study was to reveal lung condition at early exposure in addition to the other systemic harmful effects such as serum electrolyte abnormalities and renal function.

Materials and Methods

Animals and treatment

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 ± 1.0℃) under a 12 hours light/dark cycle. The rats were given only tap water for 18 hours before the experiments to defuse the effect of ionized fluoride (F) contained in the diets. All aspects of these studies were conducted following the guidelines of the Osaka Medical College 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 the massive expansion of HFA over respiratory fields. Therefore, the aerosolization was applied to the experimental model. Aerosolized particles of 16–22 μm 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. 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 1–2 mm 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.

Acute exposure to the subtoxic dose

Particles of HFA solution and saline (70 μL) were i.t. sprayed to rats using an aerosol generator as examples of inhalation exposure to HFA and saline, respectively. The purpose of this study is to investigate the recovery process within several days after inhalation exposure. In accordance with the dose-mortality curve, the dose of the HFA-treated group was set at 0.36 mg/kg body weight (0.4%, 70 μL). This dose corresponded to LD_{30} and it satisfies the requirement that aggravation of general and respiratory states should be observed, and the rats were survived for at least forty-eight hours after the exposure[7]. The blood, bronchoalveolar lavage fluid (BALF) and lung tissues were sampled 1 hr. and 48 hrs. after the i.t. administration of HFA and saline for the biological exposure and effect monitoring.

Experimental groups and sampling times

Thirty-four rats were divided into two groups according to sampling time, 1 hr. (n = 17) and 48 hrs. (n = 17). Each group time was assigned to one of two groups of exposure dose (n = 11 for HFA: n = 10 for biochemical study and n = 1 for pathological study; n = 6 for saline: n = 5 for biochemical study and n = 1 for pathological study).

Blood and BALF samples were obtained at 1hr. and 48hrs. after the i.t. administration.

Blood sampling and parameters

Blood samples were collected from the carotid artery. Many clinical reports and our previous study showed that acute electrolyte disturbance, kidney dysfunction and respiratory dysfunction occurred after acute exposure to HFA[9]. Therefore, the following parameters were measured the standard protocols recommended by assay suppliers: 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), blood urea nitrogen (BUN, urease-glutamic dehydrogenase method), creatinine (Cr, Jaffe method). 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.

Bronchoalveolar lavage fluid (BALF) sampling and parameters

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 (5 mL) was infused into the trachea (10 ml/min) and vacuumed out (1 ml/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. Supernatants were removed and 1 mL 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. Each cell solution (20 μL) was dropped onto a slide glass and dried. The cells were fixed in ethanol and Diff-Quick. One hundred cells were counted randomly and classified in terms of shape, size and color. Pulmonary surfactant including surfactant protein-D (SP-D), a complex of lipids and proteins, is secreted into the alveolar space and functions to keep alveoli from collapsing at extension[10][11]. Serum SP-D is clinically a useful biomarker for evaluating lung diseases without the need for biopsy or lavage[7]. 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[12]. Therefore, it was evaluated as a biomarker of several injury models as well as LDH in rat[13]. SP-D and LDH were measured using enzyme-linked immunosorbent assay (ELISA) technique and UV enzymatic method, respectively.

Biological exposure monitoring of HFA

The concentrations of HFA in serum and BALF were investigated on the basis of ionized fluoride (F-selective electrode method).

Sampling of lung tissues

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

Table 1 Blood gas analyses and serum ionized fluoride 1 hr. or 48 hrs. after intratracheal administration of saline or hydrofluoric acid (HFA) (0.36 mg/kg).

	Saline (1 hr.)	HFA (1 hr.)	HFA (48 hr.)
pH	7.38 ± 0.06	7.35 ± 0.07	7.36 ± 0.03
PO_2 (mmHg)	85.8 ± 32.4	50.2 ± 11.3*	68.7 ± 12.7 †
PCO_2 (mmHg)	46.2 ± 9.3	47.3 ± 11.1	45.8 ± 7.4
HCO_3^- (nmol/L)	26.5 ± 4.5	22.9 ± 7.5	25.2 ± 2.8
BE (nmol/L)	0.8 ± 1.3	− 1.2 ± 1.8	− 0.5 ± 2.0
F (μg/L)	14.4 ± 8.7	146.9 ± 56.6*	18.1 ± 6.2 †

Mean ± SD, Saline (n = 5), HFA (n = 10);
Fisher's protected LSD; *$p < 0.05$ vs. Saline, †$p < 0.05$ vs. HFA (1 hr.)

Table 2 BALF analyses 1 hr. or 48 hrs. after intratracheal administration of saline or hydrofluoric acid (HFA) (0.36 mg/kg).

	Saline (1 hr.)	HFA (1 hr.)	HFA (48 hr.)
Recovery rate (%)	90 ± 2.0	80.8 ± 3.7*	78.7 ± 3.9*
Cell count ($\times 10^5$/ml)	26.9 ± 6.5	6.5 ± 3.6*	28.4 ± 9.5 †
SP-D (ng/ml)	119 ± 27.1	83.4 ± 30.6*	401 ± 203* †
LDH (IU/L)	57.6 ± 10.8	79.6 ± 32.2*	165 ± 120* †
F (μg/L)	21.5 ± 13.3	55.4 ± 61.1	17.3 ± 20.7

Mean ± SD, Saline (n = 5), HFA (n = 10);
Fisher's protected LSD; *$p < 0.05$ vs. Saline, †$p < 0.05$ vs. HFA (1 hr.)

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

Grading of histopathological findings

Furthermore, the histopathological findings were assessed semiquantitatively, such as perivascular effusion, alveolar effusion, outgrowths and thickened bronchial epithelium, desquamation of bronchiolar epithelium and perivascular and alveolar macrophage infiltration. 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 groups were analyzed by Fisher's protected LSD. Statistical analyses were performed using SPSS® 10.0J software (SPSS Inc., Chicago, IL). Results were considered significant at $p<0.05$. There were no apparent differences in findings in blood and BALF between the saline (1 hr.) group and saline (48 hr.) group. Therefore, the following results indicated the statistical analyses using saline (1 hr.), HFA (1 hr.) and HFA (48 hr.) groups.

Results

General status

The rats showed slightly labored breaths within 1 hr. after the i.t. administration of HFA. The symptoms disappeared in the next day. All the rats survived 48 hrs. later.

Findings in blood

Table 1 shows the blood gas analyses and serum F 1 hr. and 48 hrs. after the i.t. administration of saline or HFA. The means of pH, PCO_2, HCO_3^- and BE levels showed no significant difference between the saline, HFA (1 hr.) and HFA (48 hr.) groups. The mean of PO_2 of the HFA (1 hr.) group was apparently lower than that of the saline group. That of the HFA (48 hr.) group showed a decreasing trend compared with that of the saline group, but it was higher than that of the HFA (1 hr.) group. The mean serum F level increased tenfold in the HFA (1 hr.) group compared with that in the saline group, but there were no significant differences between the saline and HFA (48 hr.) groups. The Na, K, Cl, Ca, P and BUN levels showed no significant difference between the saline and HFA groups (data not shown).

Findings in BALF

Table 2 shows the findings in BALF 1 hr. or 48 hrs. after the i.t. administration of saline or HFA. The recovery rates of the HFA (1 hr.) and HFA (48 hr.) groups were lower than that of the saline group. It showed no significant difference between the HFA (1 hr.) and HFA (48 hr.) groups. The total cell counts of the HFA (1 hr.) group were about one-fourth of that of the saline group. That of the HFA (48 hr.) group showed no significant differences compared with that of the saline group, but it was higher than that of the HFA (1 hr.) group. There were no apparent differences in the cell analysis results between both groups. The SP-D level of the HFA (1 hr.) group was lower than that of the saline group, but that of the HFA (48 hr.) group was apparently higher

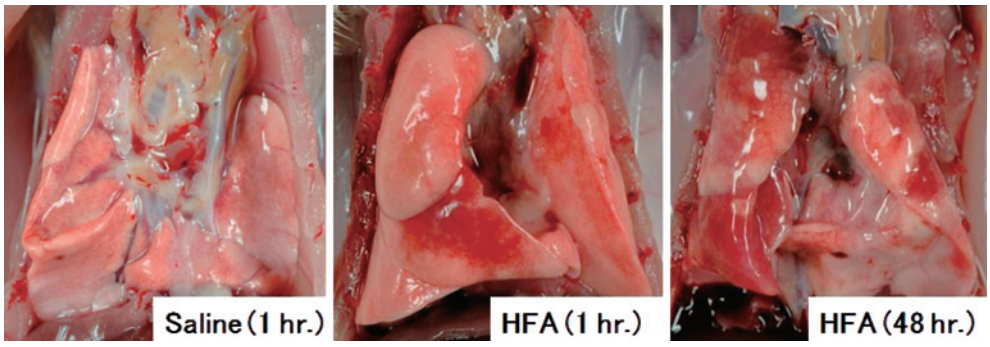

Fig. 1 Macroscopic findings of lungs after intratracheal administration of saline (1 hr.) or hydrofluoric acid (HFA) (0.36 mg/kg, 1 hr. and 48 hr.).
Diffuse swelling and intrapulmonary hemorrhage were observed in the HFA (1 hr.) group.
Slight swelling and intrapulmonary hemorrhage were observed in the HFA (48 hr.) group.

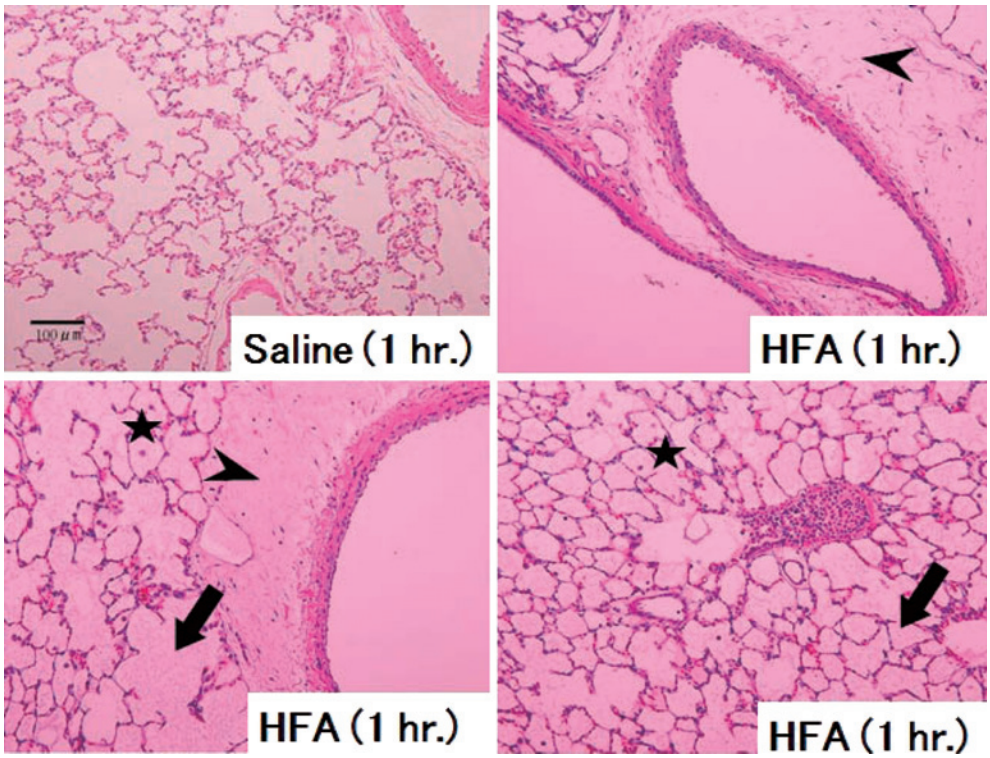

Fig. 2 Histopathological findings of lungs 1 hr. after intratracheal administration of saline or hydrofluoric acid (HFA) (0.36 mg/kg). (H & E stain). Eosinophilic proteinaceous fluid in the alveolar spaces (arrow), perivascular effusions (arrowhead) and infiltration of macrophages (asterisk) were observed in bilateral upper and lower lobes in the HFA (1 hr.) group.

than that of the saline group and HFA (1 hr.) group. The LDH level of the HFA (1 hr.) group was higher than that of the saline group, but that of the HFA (48 hr.) group was threefold higher than that of the saline group. The F level in BALF of the HFA (1 hr.) group showed an increasing trend compared with that of the saline group. It showed no significant difference between that of the saline and HFA (48 hr.) groups.

Morphological comparison

Fig. 1 shows the macroscopic findings. In the saline group, there were no degeneration, discoloration, and erosion on bilateral pulmonary surfaces. In the HFA (1 hr.) group, the bilateral lung showed diffuse swelling and intrapulmonary hemorrhage, particularly in the right lower lobe. In the HFA (48 hr.) group, there were intrapulmonary hemorrhages in the bilateral lobes and slight swelling was observed.

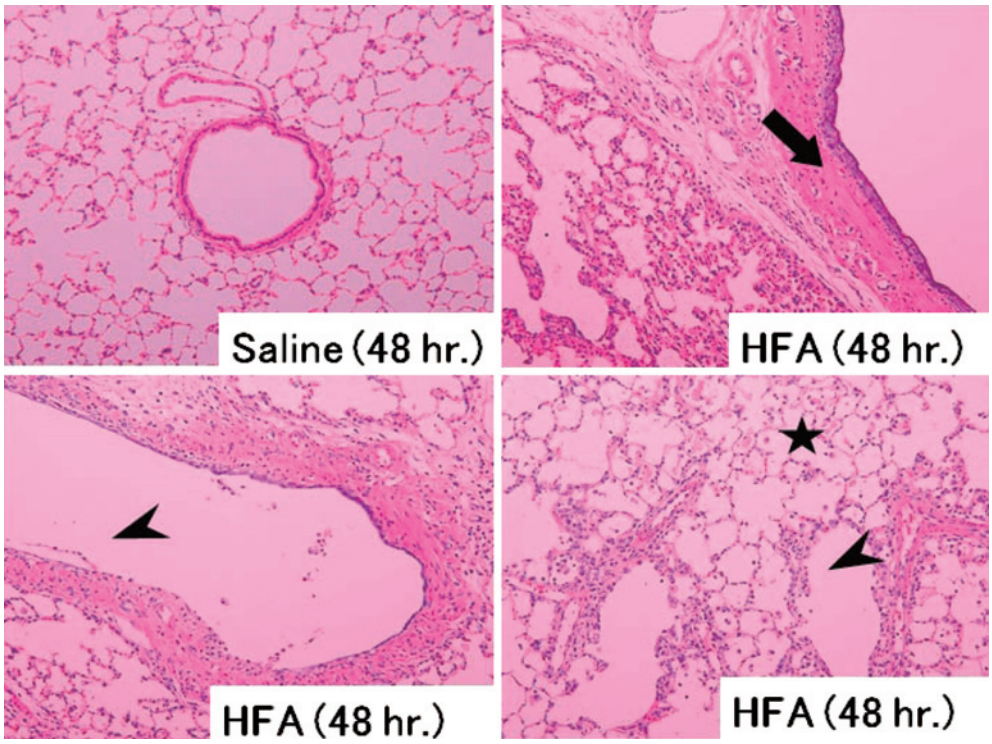

Fig. 3 Histopathological findings of lungs 48 hrs. after intratracheal administration of saline or hydrofluoric acid (HFA) (0.36 mg/kg). (H & E stain). Outgrowths and thickened bronchial epithelium (arrow), desquamation of bronchiolar epithelium (arrowhead) and perivascular or alveolar macrophage infiltration (asterisk) were observed in bilateral upper and lower lobes in the HFA (48 hr.) group.

Table 3 Semiquantitative gradings of pathohistological findings in the upper and lower lobes of bilateral lungs 1 hr. or 48 hrs. after intratracheal administration of hydrofluoric acid (HFA) (0.36 mg/kg).

	HFA (1 hr.)				HFA (48 hr.)			
	Right		Left		Right		Left	
	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Perivascular effusion	1	2	3	3	1	1	1	1
Alveolar effusion	1	2	3	3	1	1	1	1
Outgrowths and thickened bronchial epithelium	0	0	0	0	2	2	2	2
Desquamation of bronchiolar epithelium	0	0	0	0	3	3	3	3
Perivascular and alveolar macrophage infiltration	1	1	1	1	3	3	3	3

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.

Lung tissues

Fig. 2 shows histopathological findings of lungs 1 hr. after the i.t. administration of saline or HFA. In saline group, there were no disorders in the alveolar architectures such as blood vessels, alveoli, alveolar ducts, alveolar septa, terminal bronchioles, and respiratory bronchioles. Bronchiolar epithelium cells were also normally arranged. In the alveolar spaces, macrophages were slightly scattered without effusion. In the HFA (1 hr.) group, there were perivascular effusions and eosinophilic proteinaceous fluid in the alveolar spaces with infiltration of macrophages. Fig. 3 shows histopathological findings of lungs 48 hrs. after the i.t. administration of saline or HFA. In the HFA (48 hr.) group, there were outgrowths and thickness in bronchial epithelia and infiltrations of macrophages in the alveolar spaces. Bronchiolar epithelia were desquamated. There were few perivascular effusions in the alveolar spaces.

Grading of histopathological findings

Table 3 shows semiquantitative gradings of histopathological findings in the upper and lower lobes of bi-

lateral lungs 1 hr. or 48 hrs. after the i.t. administration of HFA. There were no abnormal findings in the saline group (data not shown). Perivascular effusions were mild in the right lower lobe and moderate in the left lobe in the HFA (1 hr.) group and minimal in the HFA (48 hr.) group. Alveolar effusion was mild in the lower right lobe and moderate in the left lobe in the HFA (1 hr.) group, and minimal in the HFA (48 hr.) group. Outgrowths and thickened bronchial epithelia were absent in the HFA (1 hr.) group, and mild in all the lobes in the HFA (48 hr.) group. Desquamations of bronchiolar epithelia were absent in the HFA (1 hr.) group and moderate in all the lobes in the HFA (48 hr.) group. Perivascular and alveolar macrophage infiltration were minimal in the HFA (1 hr.) group and moderate in all the lobes in the HFA (48 hr.) group.

Discussion

Our previous report indicated that pH, PO_2 (mean value 46 mmHg) and BE decreased, and PCO_2 increased significantly 1 hr. after the i.t. administration of HFA (1.92 mg/kg) and we assumed that they were caused by severe respiratory dysfunction[7]. In this study, the PO_2 (mean value 50 mmHg) decreased, but there were no significant differences in the pH, PCO_2, HCO_3^- and BE levels 1 hr. after the i.t. administration of HFA (0.36 mg/kg). Therefore, it is assumed that respiratory dysfunction was mild 1 hr. after the i.t. administration of HFA (0.36 mg/kg) compared with that of HFA (1.92 mg/kg). The mean PO_2 of the HFA (48 hr.) group showed a downward tendency compared with the saline group, but it was higher than that of the HFA (1 hr.) group. It was assumed that the hypoxemia transitorily appeared in 1 hr. and respiratory dysfunction tended to improve in 48 hrs.

The P and BUN levels of the HFA group were significantly higher than those of the saline 1 hr. group after the i.t. administration of HFA (1.92 mg/kg) in our previous study[7]. It was suggested that the P and BUN levels might be increased by tissue disruptions and hemolysis. The Na, K, Cl, Ca, P, BUN and Cr levels showed no significant differences between the HFA and saline groups in this study. Therefore, it was suggested that there were few tissue disruptions and hemolysis after the i.t. administration of HFA (0.36 mg/kg). Therefore, it was suggested that the lung injury was milder than that after the i.t. administration of HFA (1.92 mg/kg). In our previous study, the mean serum F concentration rapidly increased to 830 (μg/L) 1 hr. after the intravenous administration of HFA (1.6 mg/kg)[14], and it was 630 (μg/L) 1 hr. after the i.t. administration of HFA (1.92 mg/kg)[7]. It was assumed that the F level dose-dependently increased because it was 147 (μg/L) after the administration of HFA (0.36 mg/kg) in this study. There were no significant differences in the F level between the saline and HFA (48 hr.) groups in this study. In our previous study, the F level decreased to the preadministration value 2 hours after the intravenous administration of HFA (1.6 mg/kg)[14]. It was previously found that F clearance is dependent on renal function[15]. It has also been proposed that the serum concentration of F is decreased by the short half-life of F[16], the formation of CaF_2[17]and fluorapatite[18]. It was assumed that HFA was rapidly absorbed from lung tissues to blood and it was metabolized from the blood 48 hrs. later. Our previous study also reported the decrease in the recovery rates of BALF 1 hr. after the i.t. administration of HFA (1.92 mg/kg)[7]. Therefore, it was suggested that the decreases in the HFA (1 hr.) and HFA (48 hr.) groups were caused by alveolar abnormalities in this study.

It was reported the decreases in the total cell counts in BALF 2 hours after the inhalation of gaseous HF in human[19]and we reported decreases 1 hr. after the i.t. administration of HFA (1.92 mg/kg) in our previous study[7]. It was assumed that the total cell counts in the HFA (1 hr.) group in this study were decreased by the cytotoxicity of HFA. The total cell counts of the HFA (48 hr.) group increased compared with that of the HFA (1 hr.) group in this study. It was assumed that the inflammation reaction gradually increased in the alveoli and interstitium for two days[18]. The SP-D levels in BALF reflect alterations in alveolar compartments and epithelium[9]. It was reported in a previous study that the SP-D level apparently decreased 1 hr. after the i.t. administration of HFA (1.92 mg/kg)[7]. We hypothesized that HFA would directly dissolve the secreted SP-D and that the secretion was inhibited by the alveolar cell injury. Therefore, it was suggested that the decrease in the SP-D level in the HFA (1 hr.) group was also caused by the same elucidated in this study. On the other hand, the SP-D level of the HFA (48 hr.) group was apparently higher than that of the saline group. It was reported that the serum SP-D levels increased 4 hrs. after the i.t. administration of hydrochloric acid as a model of moderate

lung injury[13]. It was predicted that the SP-D level in BALF might be increased by a sublethal dose of HFA after several days in our previous study[7]. It was assumed that this result is one of the lines of evidence supporting our hypothesis. The LDH level in BALF of the HFA (1 hr.) group was higher than that of the saline group, but that of the HFA (48 hr.) group was threefold higher than that of the saline group. Increase in the LDH activity in BALF is useful as a rapid screening test for lung injury in response to exposure to pneumotoxic chemicals[20]. It was suspected that the lung injury increased the LDH level after 1 hr. and 48 hrs. in this study. The LDH level of the HFA (48 hr.) group was markedly higher than that of the HFA (1 hr.) group. It was assumed that the LDH level increases immediately after the exposure in a time-dependent manner. It was assumed that the F level in BALF dose-dependently increased because the means of F were 55.4 (μg/L) 1 hr. after the i.t. administration of HFA (0.36 mg/kg) in this study, and 138 (μg/L) in our previous study (HFA (1.92 mg/kg))[7]. There were no significant differences in the F level between the saline and HFA (48 hr.) groups in this study. It was assumed that the retained HFA in the lung after 1 hr. would have been metabolized from the alveolar spaces within 48 hrs.

In the HFA (1 hr.) group, the bilateral lung showed swelling. It was considered that the macroscopic swelling was mainly derived from the effusion into the alveolar spaces. The semiquantitative grading indicated that the prominent findings were the perivascular and alveolar effusion into the alveolar spaces in the HFA (1 hr.) group, and thickened bronchial epithelium and desquamations of bronchial epithelium in the HFA (48 hr.) group. The damage and restoration exist together in the lung tissue of the HFA (48 hr.) group. It was suggested that the PO_2 tends to improve in 48 hrs. with the reduction of the effusion into the alveolar spaces rather than abnormalities such as the thickened bronchial epithelium. It was considered that the respiratory condition was improved by the reduction of the alveolar effusion and the recovery of surfactant protein a few days later.

Conclusions

Acute hypoxia after HFA inhalation was observed within 1 hr. following the alveolar effusion and reduction of surfactant protein. However, a subtoxic dose could lead to an improvement of the respiratory condition, because HFA was rapidly eliminated from the lungs and the condition of the alveolar regions gradually improved within days.

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フッ酸吸入曝露による急性肺障害の早期回復過程における生化学的・病理組織学的所見の検討

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―キーワード―
フッ酸，肺胞洗浄液，エアロゾル，吸入曝露，肺障害，経時的変化

【目的】フッ酸（HFA）の吸入後の労災報告には急性致死あるいは短期回復の転帰がある．前者の病態と機序は我々が先行研究にて報告した．後者はHFAの有害作用と代謝の相互影響が推察されるが，報告は殆どない．今回，吸入曝露後の急性肺障害の発症から回復への過程を生化学的かつ病理組織学的に把握し，HFAの体内動態と関連させて検討した．

【方法】ラット（投与1時間後群（HFA 1h）・48時間後群（HFA 48h））にHFA（非致死量）と生理食塩水（対照群）をミスト化し，気管内に単回散布した．上記時間後に動脈血，肺胞洗浄液（BALF）および肺標本を採取した．

【結果】PO_2においてHFA 1hは対照群に比し低下し，HFA 48hはHFA 1hに比し上昇した．BALF中の肺胞サーファクタント蛋白（SP）の一種であるSP-Dにおいて HFA 1hは対照群に比し低下し，HFA 48hは対照群およびHFA 1hに比し上昇した．BALF中のLDHにおいてHFA 1hは対照群に比し上昇し，HFA 48hはHFA 1hに比し，さらに上昇した．血液およびBALF中のHFAの指標としてのフッ素（F）濃度においてHFA 1hは対照群に比し上昇し，HFA 48hは対照群と差がなかった．病理所見においてHFA 1hは血管周囲および肺胞腔への中等度の滲出液，HFA 48hは気管支上皮の中等度の肥厚および剝離を示した．

【考察】PO_2は投与後，急速に低下するが，徐々に改善した．HFAは肺胞内に貯留後，速やかに血中移行し，代謝された．SP-DはHFAの細胞毒性により，低下するが，HFAの肺内からの消失に伴い，LDHとともに肺組織障害に対する反応性の上昇を示した．病理所見から肺胞腔への滲出液が急速に増加し，後日消退した．以上より，非致死量の曝露であれば，一過性の低酸素血症が滲出液の減少およびSPの回復により，数日間で改善すると考えられた．

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