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Pulmonary Toxicity Induced by Intratracheal Instillation of Coarse and Fine Particles of Cerium Dioxide in Male Rats

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Abstract: In order to examine the short-, medium- and long-term effects of cerium dioxide particles of different sizes on the lung, 10-wk-old male Wistar rats were administered a physiological saline solution with a suspension of coarse or fine particles of cerium dioxide at 34 mg/kg body weight by a single intratracheal instillation. Lungs were examined with cellular and biochemical analyses of the bronchoalveolar lavage (BAL) fluid and histopathology on different days after the instillation. Geometric mean and geometric standard deviation of the diameter were 3.90 μm ± 1.93 for the coarse (Ce-C) particles, and 0.20 μm ± 1.20 for the fine (Ce-F) particles. There were no lesions in the lung in the Ce-C-instilled group at any time point after the instillation. The instillation of Ce-F particles primarily induced inflammation, granulomas, mobilization and impairment of alveolar macrophages (AMs), and pulmonary alveolar proteinosis, together with very slight degrees of Type II epithelial cell hyperplasia and of collagen deposition. The pulmonary toxicity of Ce-F-instilled rats was found to be markedly enhanced in sharp contrast to that of Ce-C-instilled rats on the basis of equal mass concentration, suggesting clear dependence of the pulmonary toxicity on numbers and sizes of particles. Causative factors for the pulmonary alveolar proteinosis are discussed with reference to the impaired AMs.

Key words: Cerium dioxide, Particles, Inflammation, Granuloma, Pulmonary alveolar proteinosis, Alveolar macrophage

Introduction

Since rare earth elements have excellent properties of magnetism and electron state[1)], industrial products containing rare earth elements are widely used in a variety of functional materials such as high-efficiency magnetic materials, solid metal hydrides and catalysts. One of rare earth metal oxides, cerium dioxide (CeO_2), is used as an abrasive for polishing mirrors and lenses, in high-temperature ceramics and metallurgy, as a constituent of carbon arc lamps to produce intense white illumination, and as an alternative to precious metals such as platinum and palladium. Especially, fine CeO_2 particles with a diameter of less than one micrometer are used for final polishing of lenses[2)] and in automotive catalytic converters[3)]. Production volume of CeO_2 in Japan is reported to have increased from 5,300 tons in 1997 to 8,200 tons in 2006[4)].

Widespread use of functional materials containing rare earth elements in manufacturing industries has raised occupational and environmental health concerns over the possibility of increased health risks of humans exposed to rare earth metals in their working and living environments. Occupational exposure to cerium and its dioxide has been reported to cause pneumoconiosis in workers[5–14)]. Experimental toxicology studies have demon-

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strated that rare earth elements including yttrium, lanthanum and neodymium cause pulmonary toxicity in rats[15–18].

The present study was designed to examine the temporal relationship of toxic pulmonary responses of male Wistar rats to coarse and fine CeO_2 particles for short-, medium- and long-terms, in order to provide basic toxicity data for health risk assessments of humans exposed to CeO_2 particles of different sizes in working and living environments. The lungs were examined for injury by cellular and biochemical analyses of bronchoalveolar lavage (BAL) fluid and for lung histopathology by light and electronmicroscopy.

Materials and Methods

Chemicals

Coarse and fine CeO_2 particles (Ce-C and Ce-F, respectively) used in the present study were purchased from Kojundo Chemical Laboratory (Sakado, Japan). The sizes of Ce-C and Ce-F particles suspended in distilled water were determined by scanning electron microscopy (SEM). The solubility of Ce-F particles in pseudo alveolar fluid was determined by a method described by Takaya *et al*[19].

Animals

The present study was approved by the Institutional Committee for Animal Care and Use of the National Institute of Occupational Health, Japan (JNIOSH), and the animals were cared for in accordance with the Institutional Guidelines for Animal Care and Use of JNIOSH. Male rats of Wistar strain (Jcl:Wistar) were obtained from Clea Japan (Tokyo, Japan) at the age of 8 wk. The animals were housed in stainless steel wire-mesh cages (5 rats/cage) in the animal colony of JNIOSH, and were quarantined and acclimated there for 2 wk. The 12-h light (8:00–20:00)/dark (20:00–8:00) cycle was automatically controlled. The colony room was maintained at a temperature of 24 ± 1°C and a relative humidity of 55 ± 5%. All the animals had free access to sterilized commercial pellet diet (CE-2, Clea Japan, Tokyo, Japan), and sterilized and filtered tap water supplied by an automatic watering system.

Particle instillation and experimental design

The Ce-C or Ce-F particles were suspended in a sterilized physiological saline solution at a concentration of 20 mg/ml, and shaken rigorously with a vortex mixer for 30 s. The amount of dose was 10 mg per animal which was equivalent to 34 mg/kg body weight. Ten-week-old male rats received the Ce-C or Ce-F-suspended solution by intratracheal instillation under inhalation anesthesia using an airflow of 4 l/min, containing 3.5% halothane. Then, an aliquot of the suspension of 0.5 ml together with 0.5 ml of air was injected into the distal end of the trachea by cannulation under dissecting-microscopic observation of the pharynx. Animals receiving a physiological saline solution of 0.5 ml and air of 0.5 ml were used as a control. Delivery of the instilled particles into the lungs with a good distribution was ensured by observing both wheezing sound of respiration with a stethoscope and rapid recovery from anesthesia with neither abnormal behavior nor negative health outcomes at the time of instillation.

The present studies consisted of a short- and medium-term study and a long-term study. In order to examine the short- and medium-term effects, the rats were sacrificed on Days 3, 7, 14, 30, 90 and 180 after the instillation. At each time point, cellular and biochemical analyses of BAL fluid were performed on 5 rats and 2 rats were examined for lung histopathology by light-microscopy. In addition, transmission electron microscopic (TEM) examination of the particle deposition and pulmonary alveolar proteinosis, and its relation to alveolar macrophages (AM), was carried out on 2 rats sacrificed on Day 18 after the instillation of Ce-C and Ce-F particles. The long-term effects were examined by lung histopathology, using 3–5 rats sacrificed at 6, 12 and 18 months after the single instillation of Ce-C or Ce-F particles.

Cellular and biochemical analysis of bronchoalveolar lavage (BAL) fluid

Five rats were euthanized with an overdose of pentobarbital sodium on each of the days noted above from Day 3 to 90 for the Ce-C-instilled group and from Day 3 to 180 for the Ce-F-instilled group. The right lungs were lavaged 3 times with 7 to 9 ml of phosphate saline, and the wash-out was collected. Total number of cells in the BAL fluid was counted with an automatic cell counter (Sysmex F-800, Toua Medical Electronics, Tokyo, Japan). The BAL fluid was centrifuged with a cytospin (Cytospin3, Shandon, USA), and the inflammatory cells were stained with May-Grünwald-Giemsa and periodic acid-Schiff (PAS). Then, the numbers of the various types of inflammatory cell were determined by observing a total of 500 cells under a light-microscope. The ratio of each type of flammatory cell were calculated by dividing the number of each inflammatory cells by the total number of cells observated (500 cells) and these ratios were then multiplied by the total number of cells in the BAL fluid to obtain the total number of inflammatory cells. BAL fluid was centrifuged at 10,000 rpm and 4°C for 10 min, and aliquots of the acellular supernatant were taken for biochemical analysis. Total protein (TP) was measured to assess the breakdown in the integrity of the

alveolar-capillary barrier, and lactate dehydrogenase (LDH) activity was measured to evaluate cytotoxicity with LDH UV Test (Wako Pure Chemicals Ind. Ltd., Japan). Monocyte chemoattractant protein-1 (MCP-1) in the BAL fluid from the Ce-F-instilled rats was measured by a sandwich enzyme-linked immunosorbent assay (ELISA), with Immunoassay Kit Rat MCP-1 (BioSource International, Inc., USA)[20].

Lung fixation and histopathology

Two rats in the short- and medium-term study and 3–5 rats in the long-term study were selected as described above for lung histopathology examination.

In order to prepare the tissues for light microscopic examination, the rats were sacrificed by exanguination from the aorta under deep anesthesia with pentobarbital sodium, and the trachea was ligated. All the rats were prepared for histopathological examination by a method described previously[21].

In order to prepare the tissues for electronmicroscopic examination, the rats from each experimental group were sacrificed on Day 18 after the instillation by exanguination from the descending aorta, and the trachea was ligated. A 0.1M phosphate buffer solution of 100 ml containing paraformaldehyde (1%) and glutaraldehyde (1.5%) was injected into the portal vein for fixation, and post-fixed with 1% osmium tetraoxide in the same buffer solution for 2 h. After dehydration in graded ethanol, about 0.5 to 0.8 mm thick slices of the lung were sectioned along the longitudinal axis of the main bronchus, and other slices were cut horizontally to the bronchial axis. After dehydration in graded ethanol, these slices were embedded in Epon resin, and thin-sectioned. Ultrathin sections of lungs were stained with uranyl acetate and lead citrate and examined by a combined light and electron-microscope (LEM: LEM-2000, Topcon, Ltd., Tokyo, Japan) and a transmission electron microscope (TEM: H-8000, Hitachi, Ltd., Tokyo, Japan).

Each histopathological lesion was scored on a scale of increasing severity from 0 to 4 by diagnostic criteria described previously[21].

Statistics

Relative weight of the left lung and the biochemical parameters of the BAL fluid were analyzed by Dunnett's multiple comparison test using statistics software (SPSS 15.0J, SPSS Japan Inc.). Differences between groups at $p<0.05$ were considered significant.

Results

Characterization of CeO_2 particles

SEM examination of particle sizes revealed that the geometric mean and standard deviation of diameters were 3.90 μm ± 1.93 for Ce-C particles and 0.20 μm ± 1.20 for Ce-F particles. The solubility of Ce-F particles in pseudo alveolar fluid was 3.3 ppb (w/w) in a 24-h period.

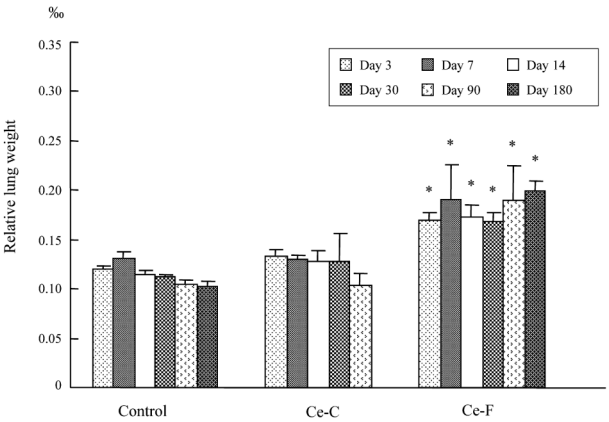

Fig. 1. Time-course changes in the relative weight of the left lung at different time points after the intratracheal instillation of coarse and fine CeO_2 particles and vehicle saline.
Bars with T indicate means and SD, *$p<0.05$ (compared to the control group).

Changes in body weight and relative lung weight

There was no statistically significant difference in body weight at any time point of necropsy between the Ce-C and Ce-F-instilled groups and the vehicle control (Data not shown). Figure 1 shows time-course changes in relative weights of left lungs on different days after the instillation between the Ce-C and Ce-F-instilled groups and the vehicle-instilled control. There was no significant difference in the lung weight at any time point between the Ce-C-instilled group and controls. On the other hand, the Ce-F-instilled group exhibited the significantly increased lung weight throughout the whole observation period up to Day 180 compared with the control group.

Cellular analysis of BAL fluid

The amount of BAL fluid retrieved was about 80% of the lavage total for the Ce-C and Ce-F-instilled groups and the control group. Gross examination of the BAL fluid from the Ce-F-instilled group revealed that the BAL fluid had an opaque, milky appearance on Days 7 to 180. Figure 2 shows numbers of inflammatory cells in the BAL fluid from the Ce-C and Ce-F-instilled groups at different time points after the instillation, as compared with those of the vehicle control group. The Ce-C-instilled group showed a transient increase only in the number of neutrophils during the first 2 wk. Microscopic observation of the inflammatory cells in the BAL fluid from the Ce-C-instilled group revealed the presence of AMs phago-

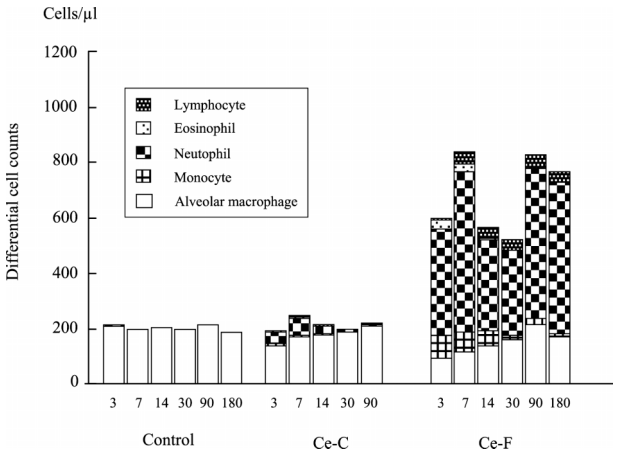

Fig. 2. Differential number of inflammatory cells in the BAL fluid from the Ce-C and Ce-F-instilled rats.
Bars indicate differential cell counts.

cytosing the Ce-C particles (Fig. 3A) without degeneration or destruction of the AMs up to Day 90.

On the other hand, marked changes in cellularity occurred in the BAL fluid from the Ce-F-instilled group throughout the whole observation period up to Day 180 (Fig. 2). These changes were characterized by a marked and persistent increase in the number of neutrophils, together with marginal increases in lymphocytes and monocytes. Initially, AMs decreased in number during the first 14 d, and then they gradually recovered toward a normal level. Particle-laden, degenerated AMs were PAS-positive, indicating accumulation of lung surfactant in the AMs (Fig. 3B).

Biochemical analysis of BAL fluid supernatant

As shown in Fig. 4, there was no significant difference in TP concentration or LDH activity in the BAL fluid supernatant at any time point up to Day 90 after the instillation between the Ce-C-instilled group and the control.

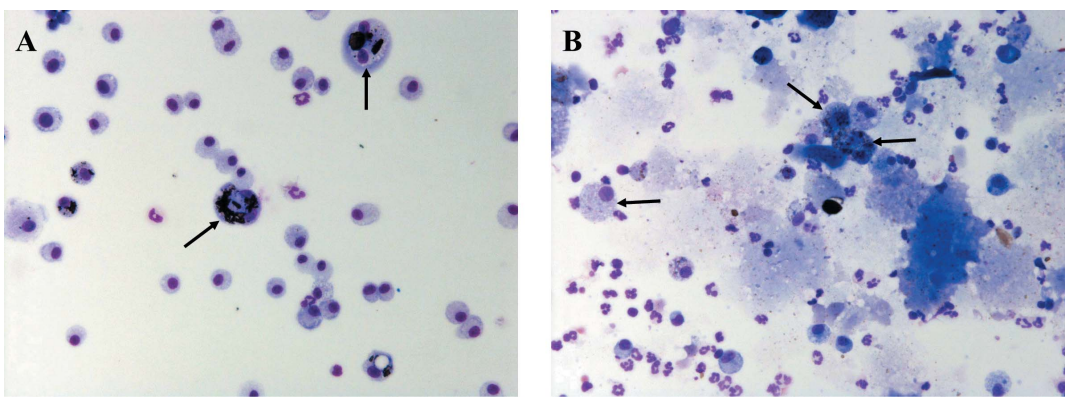

Fig. 3. Lung microphotographs showing (A) AMs phagocytosing Ce-C particles in BAL fluid, as indicated by arrows, on Day 30, and (B) neutrophils, and degeneration and destruction of AMs in BAL fluid from the Ce-F-instilled group, as indicated by arrows, on Day 180 after the instillation.
A and B were stained with May-Grünwald-Giemsa.

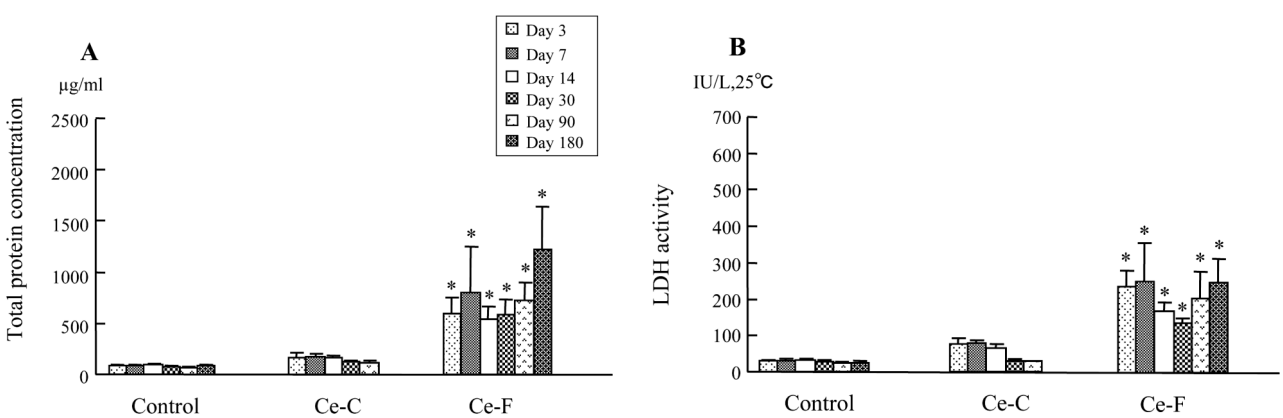

Fig. 4. Total protein concentration (A) and LDH activity (B) in the BAL fluid supernatants from the Ce-C and Ce-F-instilled and control rats.
Bars with T indicate means and SD. *$p<0.05$ (compared to the control group).

However, these two biochemical parameters in the Ce-F-instilled group were significantly increased at all time points up to Day 180 as compared with the respective control values. The means and standard deviations of MCP-1 in the acellular BAL fluid from the Ce-F-instilled group were 101 ± 78.8 pg/ml on Day 7, 135 ± 72.2 on Day 14, 345 ± 249.2 on Day 90 and 467 ± 128.1 on Day 180 after the instillation, whereas the MCP-1 value for the control group remained below the detection limit of 23.4 pg/ml.

Histopathology of the affected lung

Short- and medium-term effects: As shown in Table 1, light microscopic examination of the lungs from the Ce-C-instilled group revealed that pulmonary lesions did not occur in the short and medium terms, except for agglomerated particles surrounded by the epithelial cells and a few AMs in the alveolar ducts at all the different time points up to Day 180 (Fig. 5A).

On the other hand, lung histopathology of the Ce-F-instilled group was characterized by the presence of agglomerated Ce-F particles around the alveolar duct and neighboring alveoli and by induction of inflammation, small granulomas (Fig. 5B), mobilization and destruction of AMs, and pulmonary alveolar proteinosis. The acute inflammation was high during the first week but tended to recover. The granuloma persisted throughout the whole observation period up to Day 180. Mobilized and disruptive AMs that phagocytosed the Ce-F particles and lung surfactant were noted in the alveoli. Both the degenerative AMs and deposition of the lung surfactant continued to develop until Day 180. Notably, pulmonary alveolar proteinosis occurred on Day 14, and its severity was gradually enhanced with the elapse of time after the instillation. No collagen deposition in the bronchiolar and alveolar ducts or in the alveoli was observed in the lungs from the Ce-C and Ce-F-instilled groups.

Long-term effects: No histopathological lesion was observed in the lungs from the Ce-C-instilled group throughout the whole observation period of 18 months, except for agglomerated particles surrounded by epithelial cells and a few AMs in the alveolar ducts.

On the other hand, the Ce-F-instilled group exhibited persistent mobilization and destruction of AMs and pulmonary alveolar proteinosis (Fig. 5C) in the alveoli over the long-term. The granulomas occurring in the short- and medium-terms disappeared in the long-term. A very slight degree of Type II epithelial cell hyperplasia persisted from Day 30 through 18 months. Notably, very slight collagen deposition occurred only in the bronchiolar and alveolar duct of the Ce-F-instilled group at the end of the 18-month observation period.

TEM observation: An image (Fig. 6A) of a lung from the Ce-F-instilled group sacrificed on Day 18 reveals that there were large-size, degenerative AMs that were phagocytosing the Ce-F particles and cellular debris. TEM examination of the lungs from the Ce-F-instilled group revealed that the proteinaceous materials together with the Ce-F particles were agglomerated in the alveolar space. The materials were identified as the lung surfactant,

Table 1. Averaged severities of short- and medium-term and long-term lung lesions in the rats given Ce-C or Ce-F particles by intratracheal instillation and sacrificed at different time points

Group	Days after intratracheal instillation										
	Ce-C			Ce-F							
	Short- and medium-term	Long-term		Short- and medium-term						Long-term	
	3–180 d	1 yr	1.5 yr	3 d	7 d	14 d	30 d	90 d	180 d	1 yr	1.5 yr
Bronchiole and Alveolar duct											
Inflammation	0	0	0	3	2	1	0	0	0	0	0
Granuloma	0	0	0	1	1	1	1	1	0	0	0
Deposition of collagen	0	0	0	0	0	0	0	0	0	0	1
Alveolus											
Mobilization/Disruption of AMs	0/0	0/0	0/0	2/0	3/2	3/2	3/2	3/2	3/2	3/3	3/3
Inflammation	0	0	0	1	3	2	1	1	1	0	0
Pulmonary alveolar proteinosis	0	0	0	0	0	1.5	2	3.5	4	4	3
Granuloma	0	0	0	1	1	1	1	1	1	0	0
Hyperplasia of type II epithelium	0	0	0	0	0	0	1	1	1	1	1
Deposition of collagen	0	0	0	0	0	0	0	0	0	0	0

0: Negative, 1: Very slight, 2: Slight, 3: Moderate, 4: Marked.
The scored severity was averaged over the two rats for the short- and medium-term studies and over the 3–5 rats for the long-term study.
AMs: Alveolar macrophages.

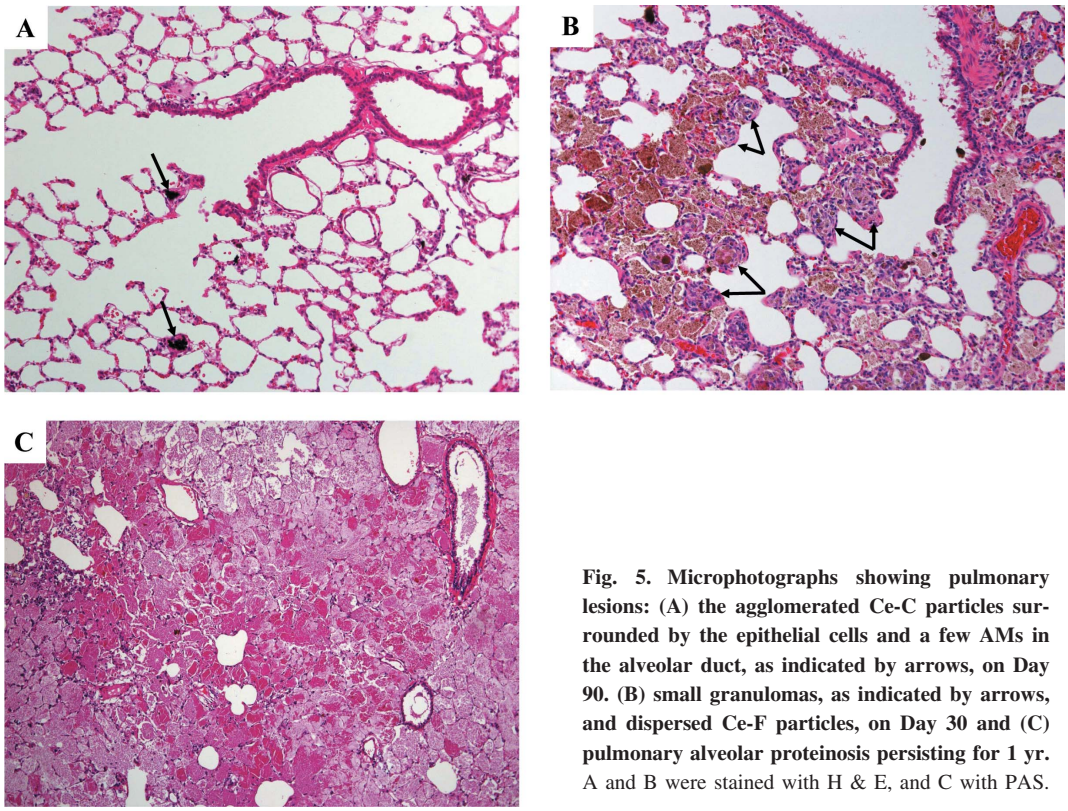

Fig. 5. Microphotographs showing pulmonary lesions: (A) the agglomerated Ce-C particles surrounded by the epithelial cells and a few AMs in the alveolar duct, as indicated by arrows, on Day 90. (B) small granulomas, as indicated by arrows, and dispersed Ce-F particles, on Day 30 and (C) pulmonary alveolar proteinosis persisting for 1 yr. A and B were stained with H & E, and C with PAS.

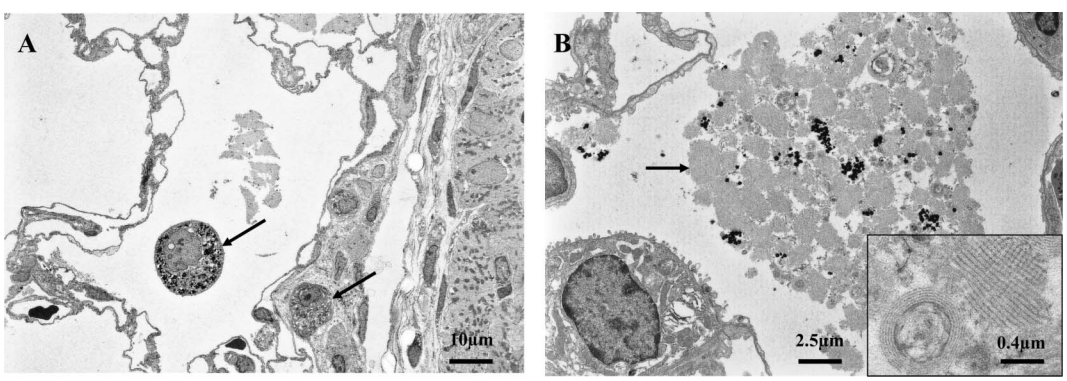

Fig. 6. TEM images of the lung of a Ce-F-instilled rat sacrificed on Day 18 after the instillation.
(A) AMs phagocytosing Ce-F particles in the alveolar space and alveoli as indicated by arrows. (B) Presence of dispersed Ce-F particles and accumulation of the lung surfactant in the alveolar space as indicated by an arrow. The inset shows a TEM image of the multilamellated, lattice-shaped and myelin-like structure of the lung surfactant.

because of its multilamellated, lattice-shaped and myelin-like structure (Fig. 6B). The LEM examination also confirmed that no proteinaceous material was found in the pulmonary alveoli of the Ce-C-instilled group.

Discussion

It was found in the present study that intratracheal instillation of Ce-F particles primarily induced inflammation, granulomas, mobilization and impairment of AMs, and pulmonary alveolar proteinosis. The acute and subchronic inflammatory responses to Ce-F particles were characterized by persistent elevations of neutrophils and foamy and degenerative AMs phagocytosing the fine particles, together with marginal increases in the numbers of lymphocytes and monocytes. In contrast, Ce-C particles did not elicit any inflammatory responses or associated histopathological lesions except for the persistent occurrence of agglomerated particles surrounded by epithelial cells and a few AMs in the bronchiolar and alveolar ducts

throughout the whole observation period of 18 months, and this was considered as evidence of a foreign body reaction that may not have toxicological relevance. Consequently, it can be concluded that the pulmonary toxicity of CeO_2 particles depends clearly upon the size of CeO_2 particles and some other factors associated with the reduction of particle size.

Several causative factors might be involved in the pathogenesis of the size effect of CeO_2 particles on the toxic pulmonary responses. These factors include the number of particles and their sizes and surface areas per unit mass, and the solubility and reactivity of particles. It has been recognized that the number of particles and their surface area per unit mass markedly increase with a decrease in particle size[22, 23], and that an increased number of low-toxicity, low-solubility particles, and associated increase in the surface area per unit mass, play an important role in enhanced toxic pulmonary responses to ultrafine particles[22–28]. Thus, the present findings can be accounted for in terms of the increases in the number of particles and surface area per unit mass on the basis of several studies[24–28] which have demonstrating that ultrafine particles of low-toxicity and low-solubility such as carbon black, titanium dioxide and polystyrene are more inflammogenic in the rat lung than larger respirable particles of the same chemical composition. Indeed, Duffin *et al*.[28] suggested that the severity of inflammatory responses to low-toxicity, low-solubility particles is linearly related to the surface area of the dose instilled but not to the mass of the dose instilled. The solubility of Ce-F particles in pseudo alveolar fluid was found to be only 3.3 ppb (w/w) in a 24-h period in the present study, while the solubilities of Ce-C particles in pure water and 1% NaCl solution were 2.1 ppb and below the detection limit in the 4-d period, respectively, in our previous report[29]. Taken together, these solubility results indicate that the concentration of solubilized CeO_2 from the coarse and fine particles in the lung fluid might be so low as to have no hazardous effect on the macromolecules of the lung surface. In comparison to low-toxicity, low-solubility particles, however, particles of high-toxicity and low-solubility were reported to exhibit greater surface reactivity according to the finding by Duffin *et al*.[28], who demonstrated that ultrafine DQ12 quartz had much more inflammogenicity *in vivo* and *in vitro* than in the aluminium lactate-treated, surface-modified DQ12 quartz of the same surface area per mass. Therefore, it can be inferred on the basis of these reports[23–28] that the toxic pulmonary responses induced by the Ce-F but not by Ce-C particles are primarily attributable to the increases in the number of Ce-F particles and surface area per unit mass rather than the surface reactivity or solubility of the particles.

The present result of the Ce-F-induced pulmonary alveolar proteinosis is comparable with the previously reported findings regarading other particulate matters that intratracheal instillation of crystalline quartz of respirable sizes in rats induces prominent pulmonary alveolar proteinosis together with foamy and degenerative AMs[30], and that pulmonary alveolar proteinosis is also induced in rats by intratracheal instillation of nickel fumes and nickel oxide powder[21] and by inhalation exposure to ultrafine metallic nickel[31].

In the present study, excessive precipitation of proteinaceous materials in the alveolar space was diagnosed as pulmonary alveolar proteinosis, since these materials were PAS-positive and also exhibited a multilamellated, lattice-shaped and myelin-like structure in TEM images of the lungs from the Ce-F-instilled group. The possible involvement of surfactant catabolism and clearance by AMs in the pathogenesis of pulmonary alveolar proteinosis has been reported[32]. Since degeneration and impairment of particle-laden AMs resulting in accumulation of the lung surfactant were pronounced in both the BAL fluid and lungs from the Ce-F-instilled group in the present study, we consider the Ce-F-induced pulmonary alveolar proteinosis to be causally related to impairment of the lung surfactant metabolism due to defective AMs. Dranoff *et al*.[33] reported occurrence of pulmonary alveolar proteinosis and markedly increased accumulation of lung surfactant in AMs in the granulocyte macrophage colony stimulating factor (GM-CSF)-deficient knockout mouse, suggesting that defective AM processing of the lung surfactant, resulting from the absence of GM-CSF, is involved in the pathogenesis of pulmonary alveolar proteinosis. Taking into consideration the reported findings[34] of a marked elevation of MCP-1 in BAL fluid from patients with pulmonary alveolar proteinosis together with the possible impairment of the macrophage function and the survival, it can be inferred that the marked increase in MCP-1 levels in the BAL fluid from the Ce-F-instilled group is closely associated with defective AMs, resulting from enhanced phagocytosis of fine CeO_2 particles in the alveolar space.

It is known that pneumoconiosis is caused by occupational exposure of workers to industrial and commercial products containing cerium or its oxidized form[5–14], and that cerium-induced pneumoconiosis is pathologically characterized by fibrosis, emphysema, sclerotic thickening of septal tissue, bronchial ectasia, granulomas and chronic inflammatory peribronchial infiltrates[5–14]. It is interesting to note in the present study that some of these disorders occurring in the cerium-exposed workers, including inflammation and granulomas, were also observed in the lungs of male rats exposed to Ce-F particles by intratracheal instillation. There are differences

in route of exposure to cerium, its amount of dose and duration of exposure between the male rats used in the present study and cerium-exposed workers, in addition to a distinct species difference between rats and humans. However, Henderson *et al.*[35] demonstrated that the relative lung toxicity potentials of TiO_2 and quartz particles needed to elicit the inflammatory responses and granulomatous lesions could be appropriately evaluated using either intratracheal or inhalation exposures. Therefore, the present findings of toxic pulmonary responses of male rats to intratracheally instilled, coarse and fine CeO_2 particles would provide novel information about the hazards of lung toxicity, which could serve as a health risk assessment for humans exposed by inhalation to CeO_2 particles. It should be pointed out that the amount of dose (10 mg/rat) for fine CeO_2 particles used in the present study seems to be high and may be overloaded on the lung as compared with the estimated amount of cerium dioxide particles to which workers are exposed by inhalation in occupational settings. Further studies with lower doses of fine CeO_2 particles will be needed to delineate the dose-response relationships including the no observed effect level for the pulmonary toxic responses.

Conclusion

Single intratracheal instillation of fine CeO_2 particles in male rats primarily induced inflammation, small granulomas, mobilization and impairment of AMs and pulmonary alveolar proteinosis in the lung, whereas the coarse CeO_2 particles did not elicit any deleterious effect. Very slight degrees of Type II epithelial cell hyperplasia and collagen deposition were also noted in the fine CeO_2 particle instilled rat. We consider the markedly enhanced pulmonary toxicity of fine CeO_2 particles to be attributable to increases in the number of particles and the surface area per unit mass concentration.

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