Neurotransmitters

Fig. 2A shows the concentrations of norepinephrine, dopamine and serotonin in the PFOS-treated rat brain. Fig. 2B shows the concentrations of glutamic acid, glycine and GABA in the same tissue. The dose of PFOS was set to 250 mg/kg, which caused convulsion in both rats and mice. However, PFOS did not affect the concentrations of these neurotransmitters in the brain.

Glutamic acid oxidase activity was not affected by PFOS *in vitro* up to the concentration of 250 μg/ml (data not shown).

DISCUSSION

We could not detect neurotoxic signs of PFOS by usual observational methods. However, ultrasonic stimulus caused tonic convulsions in the PFOS-treated animals. Although ultrasonic stimulus has a convulsive effect by itself, it requires long exposure to high sound pressure to cause convulsions in animals (Commissaris *et al.*, 1998). Under the conditions of our experiment, the ultrasonic stimulus never caused convulsions or excitation in the control mice and rats, and the statistical analysis indicated a significance of the effect of PFOS. Therefore, PFOS has a proconvulsive effect on animals, while PFOA is not suggested to have any neurotoxic effect.

The convulsions were observed 24 (mice) or 36 (rats) hr after exposure to PFOS or later. This may relate to the increasing tendency of PFOS in the brain after exposure (Fig. 1). PFOS may be redistributed from the other tissues to the brain, whose PFOS concentration was relatively low.

The minimum dose of PFOS that caused convulsions was 250 mg/kg for rats and 125 mg/kg for mice, but the difference between rats and mice was not significant because only 2 or 3 animals were used for each experimental group. Therefore, subsequent experiments were conducted only for the effects of PFOS on rats.

Convulsions are caused by various central nervous system stimulants or toxins. The mechanism of such convulsants involves the effects on neurotransmitters in many cases. For example, strychnine causes tonic convulsions by competing with the inhibitory neurotransmitter, glycine. Tetanospasmin, a neurotoxin produced by *Clostridium tetani*, suppresses the release of GABA or glycine from the terminal of inhibitory nerves. Picrotoxin and pentetrazol act as antagonists for GABA receptors. Kainic acid, an agonist for glutamic acid receptors, also induces convulsions. We determined 6 kinds of neurotransmitters in rats exposed to the convolsive dose of PFOS (250 mg/kg); however, there were no significant changes in their concentrations in the brain. It was also confirmed that PFOS does not induce any neurotoxicity in either the cerebrum and cerebellum at histopathological examination. Convulsive effect of PFOS may not be attributed to the quantitative alterations of neurotransmitters or lesions of nerve cells in the brain.

Nishikawa *et al.* (2004) found that PFOS induces backward swimming of paramecia at a concentration of 20 μM (= 10 mg/l) or higher. As the swimming direction of par-

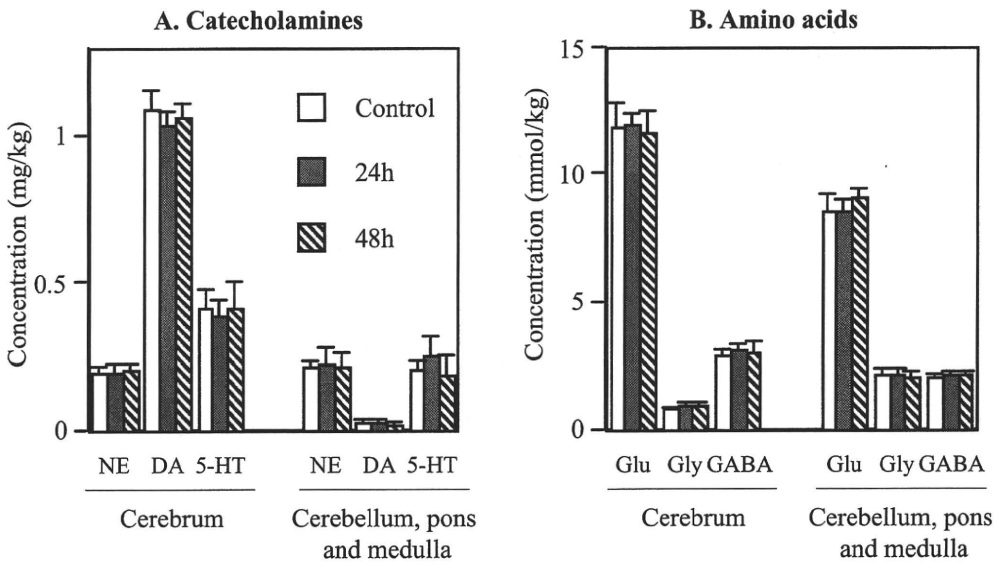

Fig. 2. Neurotransmitters in the rat brain exposed to PFOS (250 mg/kg, p.o.)

amecia is directly regulated by the concentration of intracellular Ca^{2+} (Naitoh, 1966), the induction of backward swimming indicates the increase of Ca^{2+} influx into the cell. Kawamoto *et al.* (2008) confirmed in paramecia that 3 μM or more PFOS causes initial transient depolarization followed by transient hyperpolarization and sustained depolarizarion which may trigger Ca^{2+} influx through the voltage-gated Ca^{2+} channels. Harada *et al.* (2005) have also reported that PFOS (10 and 20 μM) affects the action potential and L-type Ca^{2+} current in mammalian cells. PFOS concentrations in the PFOS-exposed rat brain were comparable to those *in vitro* where these electrophysiological effects were observed. Therefore, induction of Ca^{2+} influx into nerve cells might be involved in the convulsive effect of PFOS.

Although the PFOS-induced convulsion was observed at relatively high doses, the convulsion was never caused by a very similar perfluorochemical, PFOA. Thus, the finding of PFOS-specific convulsion may be useful to characterize perfluoro-congeners.

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Industrial Health 2010, 48, 452–459

Original Article

Characteristics of Multiwall Carbon Nanotubes for an Intratracheal Instillation Study with Rats

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Received July 23, 2009 and accepted December 3, 2009

Abstract: Much concern has been raised over the health consequences of workers exposed to carbon nanotubes. In order to characterize multi-wall carbon nanotubes (MWCNT) suspended in a phosphate-buffered saline containing 0.1% Tween 80 for an intratracheal instillation study. Length and width distributions of the MWCNT fibers, dispersion of MWCNT in the suspension and in the lung tissue and the MWCNT contents of metal impurities were investigated. Arithmetic mean length and width of the MWCNT fibers as measured on scanning electron microscope (SEM) photographs were 5.0 μm and 88 nm, respectively, and fibers longer than 5.0 μm were 38.9% of all fibers measured. Dynamic light scattering size measurement revealed that 5-min ultrasonication, together with addition of Tween 80 into the suspension, decreased the hydrodynamic diameters of the agglomerated MWCNT to those of finer particles below 1.0 μm. SEM observation showed good dispersion of MWCNT in the suspension, and in the alveoli on Day 1 after instillation. Concentration of iron, chromium and nickel in the MWCNT were 4,400, 48 and 17 ppm (wt/wt), respectively, all of which were below levels that would elicit positive pulmonary toxic responses to these metals. The results suggest that well-dispersed, long and thin MWCNT fibers exhibit asbestos-like pathogenicity in the lung.

Key words: Multiwall carbon nanotube, Metal impurities, Iron, SEM, Dynamic light scattering, Size distribution, Dose characterization

Introduction

In this decade, one of the most important developed in industrial technology is nanotechnology which makes use of nano-scale (<100 nm) structure-controlled materials. Use of nanotechnology is expected to grow exponentially in the first half of the 21st century. The growth of nanotechnology industries has prompted a rapid increase in amounts and kinds of nanomaterial production. However, the increased use of nanomaterials may result in new types of health risks for workers exposed to nanomaterials.

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At present, health hazards of nanomaterials have not been clarified sufficiently yet, and many researchers have been extensively investigating biological responses to nanomaterials, using *in vivo* and *in vitro* techniques. Among the many types of nanomaterials, carbon nanotubes (CNT) have raised much attention and concern, since their fibrous forms are similar to those of asbestos. Recent studies by Takagi *et al.*[1)] and Sakamoto *et al.*[2)] showed that mesotheliomas are induced by intraperitoneal and intrascrotal administrations of multiwall carbon nanotubes (MWCNT) in p53 gene-deficient mice and male Fischer 344 rats, respectively. Poland *et al.*[3)] also demonstrated the asbestos-like pathogenicity of long MWCNT fibers administered to the peritoneal cavity of female mice. Since inhalation is the primary

route of exposure of workers to carbon nanotubes, and since the target organ is the respiratory system, use of an inhalation exposure system would be recommended for a study of experimental toxicology. Because of the technical difficulty in generating stable MWCNT aerosol at a constant concentration for an inhalation study, the technique of intratracheal instillation or pharyngeal aspiration has been extensively used to examine the pulmonary toxicity of nanomaterials in spite of the limitations of intratracheal instillation[4]. Since MWCNT is agglomerated in a rope-like structure and insoluble in aqueous solutions, use of well-dispersed MWCNT fibers suspended in an aqueous solution containing a dispersant is a prerequisite in an intratracheal instillation study evaluating the pulmonary toxicity of MWCNT. Indeed, Mercer *et al.*[5] reported that exposure of mice to well-dispersed single-wall carbon nanotubes (SWCNT) fibers by pharyngeal aspiration affected pulmonary distribution and responses. It has also been reported that in addition to the size, shape and agglomerated state[3, 6], the content of iron used for catalysis[7–9] modified the pulmonary responses of rats and mice to CNT.

In order to evaluate the pulmonary responses of rats to MWCNT by administered intratracheal instillation, information about the characteristics of MWCNT such as length and width of MWCNT fibers, dispersion of MWCNT in the suspension and in the lung tissue and metal impurities in MWCNT is of prime importance. Therefore, the present study measured the length and width of dispersed MWCNT fibers in a suspension by scanning electron microscopic (SEM) observation, to explore how to well-disperse MWCNT fibers in a suspension and in lung tissues, and to determine concentrations of metal impurities in MWCNT that might modify the pulmonary responses to MWCNT. The results of the pulmonary toxic responses of male Fischer 344 rats to the intratracheally instilled MWCNT under the same conditions of dosage as those obtained in the present study were submitted to Industrial Health as a separate paper[10].

Materials and Methods

Test substance

MWCNT used in the present study was kindly supplied by Mitsui & Co., Ltd. (MWCNT-7, Lot No. 061220, Tokyo, Japan). This MWCNT was synthesized by the vapor phase chemical vapor deposition (CVD) and graphitization following CVD. Most manufacturers with large-scale production of MWCNT have adopted vapor phase CVD according to their published information[11, 12]. The MWCNT used in the present study was a typical sample of MWCNT being manufactured at present.

Size distribution and dispersion of MWCNT in the suspension

MWCNT was suspended in phosphate-buffered saline (PBS) containing 0.1% Tween 80 as a dispersant. Then, the suspension was subjected to ultrasonication with an ultrasonic homogenizer (VP-30S, 20 kHz, 300 W, TAITEC Co., Ltd, Tokyo, Japan) for various periods of time.

The size distribution of MWCNT in the suspension was evaluated by both SEM observation and dynamic light scattering size (DLS) measurement. Sample preparation for SEM observation of fibers was described in detail in our previous paper[13]. Briefly, a water drop of MWCNT suspension at 533 μg/ml was put on a polycarbonate membrane filter (Isopore, Millipore, MA, USA) set on a suction filtration apparatus. A drop of the sample suspension was put onto another pre-settled water-drop, and the suction was started. The filtered membrane filter samples were dried in air. After drying the filter was pre-coated with Pt-Pd for electron charge avoidance, and the MWCNT fibers were observed with a field emission SEM (S-4700, Hitachi, Tokyo, Japan). Several fields of viewing were photographed at magnifications of × 1,000 and × 5,000 for measurement of length and width, respectively. The length and width of MWCNT fibers, almost all of which appeared to be isolatably thin in each field, were measured with a curvimeter and scale loupe on enlarged photoprints. The number of fibers used in the length and width measurements were 1,000 and 500, respectively. The DLS measurement with a Zetasizer Nano DLS analyzer (Malvern, Worcestershire, UK) was performed on the PBS-Tween 80 suspension containing MWCNT at 533 μg/ml. In order to prevent excessive lung burden of MWCNT, we chose the level of 533 μg/ml which is equivalent to an intratracheally instilled dose of 160 μg/rat, based on Morrow's report[14] that the level of dust burden causing lung overload was greater than 1–2 mg of persistently retained dust in the lungs of F344 rats.

Dispersion of MWCNT in the aerosolized suspension and in the lung

In order to observe the dispersed state of MWCNT fibers in the PBS-Tween 80 suspension exactly at the time of intratracheal instillation, the suspension containing MWCNT at 533 μg/ml was ejected into the air from the tip of the microspray cannula of an Intratracheal Aerosolizer (1A-1B, PennCentury, Inc., USA) which was connected to a syringe pre-filled with the MWCNT suspension. The experimental set-up for aerosolization in a glove-box is presented in Fig. 1. The aerosol-

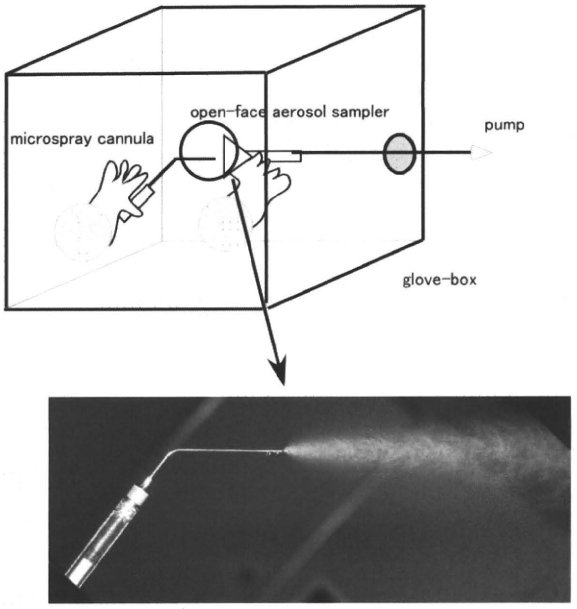

Fig. 1. The experimental set-up was installed in a glove-box (A). Aerosolized MWCNT suspension was ejected into the air from the tip of a microspray cannula which was connected with a syringe filled with the MWCNT suspension (B). The concentration of MWCNT suspension used was 533 μg/ml (equivalent to a dose of 160 μg in 0.3 ml suspension).

ized MWCNT suspension was collected on a 25-mm Isopore-membrane filter using an open-face aerosol sampler which was connected to a sampling pump (Lelandpump, SKC, USA). The sampling rate was 9 l/min, and the distance between the filter sampler and the tip of the microspray cannula was about 20 cm. The filter sample was dried in air, and after drying the filter was pre-coated with Pt-Pd. The sample was observed with the SEM.

In order to examine whether the well-dispersed state of the MWCNT fibers after intratracheal instillation was maintained in the lung tissues, the deposition of MWCNT in left lung tissues of the rats which received intratracheal instillation at a dose of 160 μg/rat was examined by SEM one day 1 after instillation[10].

Analysis of metal impurities in MWCNT

At first, the MWCNT was analyzed qualitatively for metals by Laser-Abrasion Inductively Coupled Mass Spectrometry (LA-ICP-MS). Figure 2 shows the procedure of sample preparation for LA-ICP-MS analysis. MWCNT was suspended in ethanol, and a portion of the suspension was put onto a PTFE membrane filter (Omnipore, Millipore, Billerica, MA, USA). The filter sample was fixed to a slide glass (Matsunami Glass Industry, Kishiwada, Japan) using ultraviolet curing glue (Three Bond 1771E, Three bond, Hachioji, Japan), and then pressed with 500 g weight. The fixed particle samples were vaporized by a 213 nm NdYAG laser (UP-213 Electro Scientific Industry, Portland, OR, USA) and the vapor was introduced into an Inductively Coupled Plasma-Mass Spectrometer (Agilent 7500c, Yokogawa Analytical, Tokyo, Japan) for qualitative analysis of the elements.

After the qualitative analysis, the detected metal impurities were analyzed quantitatively by graphite furnace atomic absorption spectrometry (AAS) as follows. About 20 mg of MWCNT was weighed, then the sample was digested with 5 ml of hydrochloric acid, 2.5 ml of nitric acid and 5 ml water solution for 30 min heating at 160°C. After cooling, the solution was diluted by 0.5% nitric acid to 50 ml. The metal concentrations in the sample solution were measured by a graphite atomic absorption spectrometer (Z-5010, Hitachi, Tokyo, Japan).

Results

Length and width of MWCNT fiber

Figure 3 shows the distribution of lengths and widths of MWCNT fibers as determined by the SEM analysis. The arithmetic mean and SD of the fiber lengths were 5.0 ± 4.5 μm, ranging from 0.5 μm at the minimum to 21.8 μm at the maximum. The fibers with lengths greater than 5 μm were 38.9% of fibers measured, indicating that the distributions of the lengths was skewed with longer fibers. The arithmetic mean and SD of the fiber width were 88 ± 5 nm, ranging from 40 nm at the minimum to 173 nm at the maximum.

Dispersion of MWCNT fibers in the suspension and in the lung tissue

In order to facilitate dispersion of the MWCNT fibers, Tween 80 was added at a concentration of 0.1% in the suspension in the present study. Figure 4 shows the effect of ultrasonication on MWCNT particle sizes evaluated by the DLS measurement for the hydrodynamic diameter which was numerically derived from optical measurement of the Brownian motion of the target particles, on the assumption that the particles were spherical. Five minutes ultrasonication of the MWCNT suspension was found to completely eliminate the second peak with a diameter of 7.0 μm, decrease the diameter of the main peak from 2.0 μm to below 1.0 μm, and create a new fraction of MWCNT of 0.2 μm in diameter. A SEM image indicating good dispersion of the MWCNT fibers in the suspension is presented in Fig. 5A. In the simulation experiment (Fig. 1), the microspray cannula was found to completely aerosolize

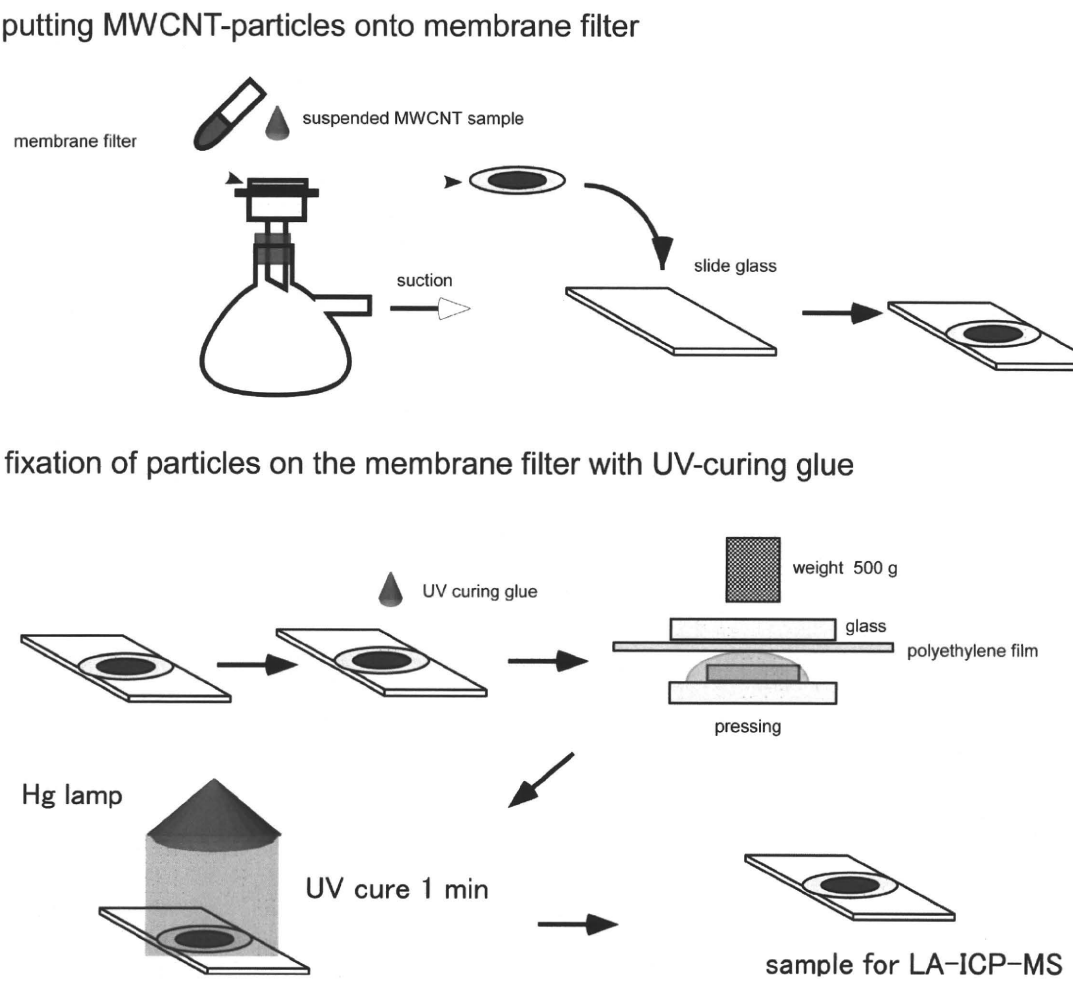

Fig. 2. A schematic diagram of the procedure of the sample preparation for the LA-ICP-MS analysis.

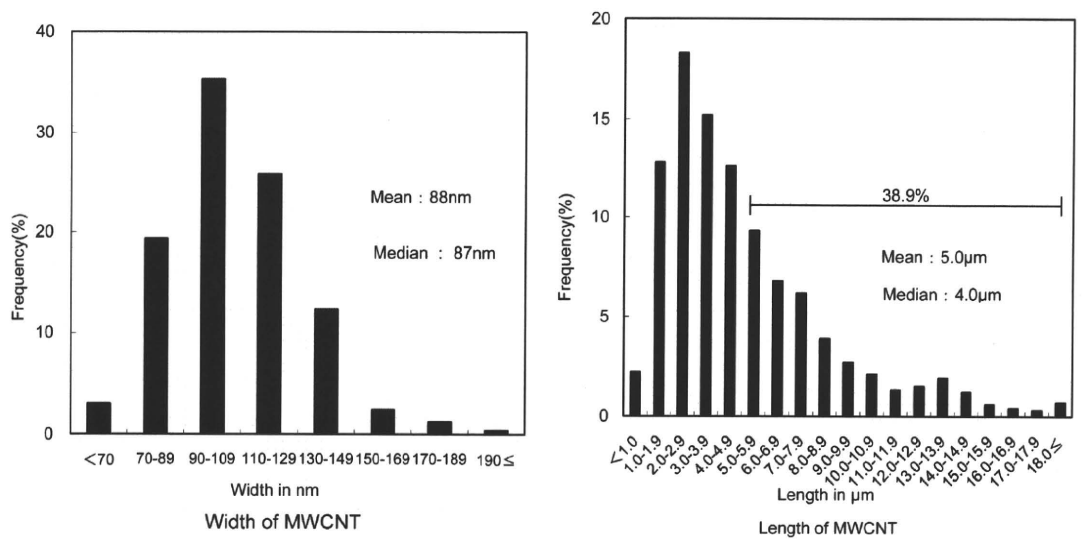

Fig. 3. Histograms of length (A) and width (B) of MWCNT fibers as measured with a curvimeter and a scale loupe, respectively, on the enlarged photographs of SEM image.

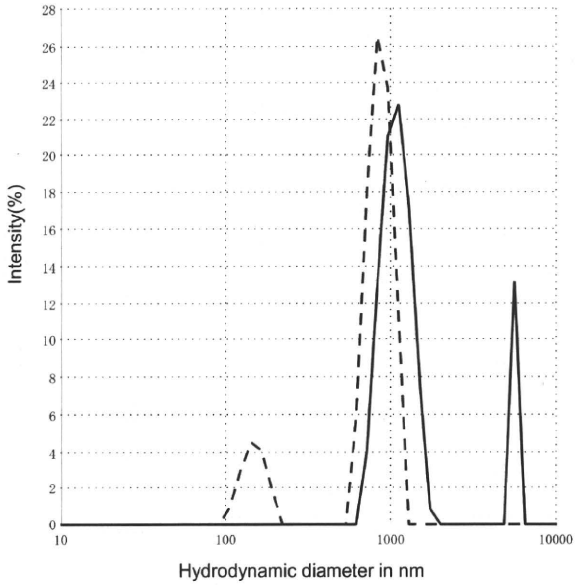

Fig. 4. Effect of ultrasonication on dispersion of MWCNT in the PBS suspension in the presence of 0.1% Tween 80 as a dispersant.
The concentration of MWCNT in the suspension was fixed at 533 μg/ml. The solid line indicates the suspension without ultrasonication, while the dashed line represents the sample ultrasonicated for 5 min.

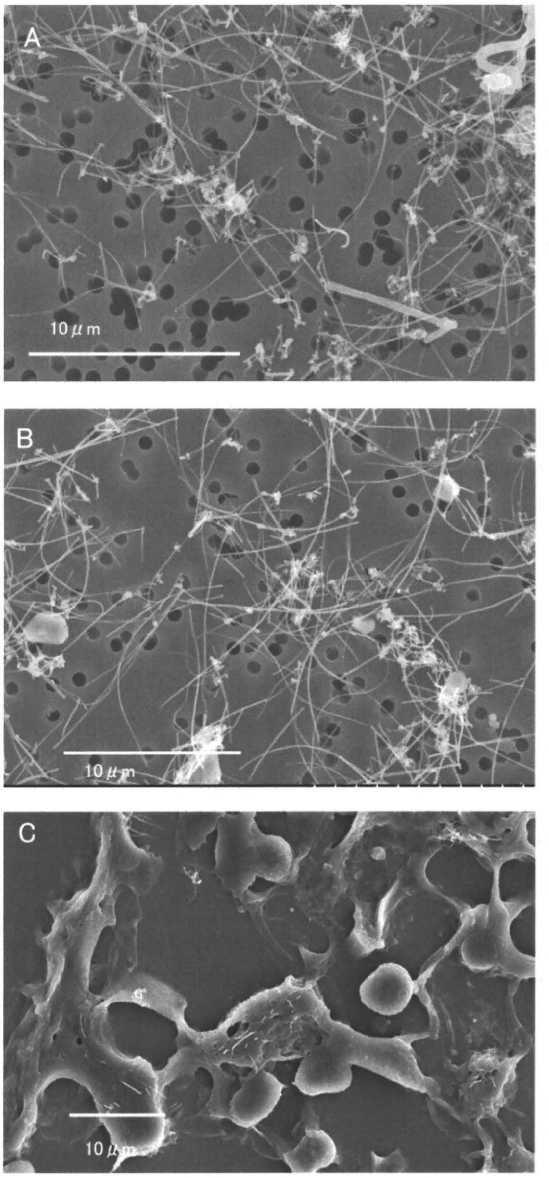

Fig. 5. SEM images of the MWCNT fibers, in the PBS-Tween 80 suspension, showing good dispersion of the fibers in the PBS-Tween 80 suspension after the 5-min ultrasonication (A), in the aerosolized MWCNT suspension ejected into air from the tip of the microspray cannula used for intratracheal instillation (B), and in the alveolar macrophages incompletely engulfing the well-dispersed MWCNT fibers in the alveoli of a rat lung on Day 1 after instillation of 160 μg MWCNT (C).
The concentration of MWCNT suspension used here was 533 μg/ml for samples A and B.

the MWCNT suspension without forming droplets at the tip. Figure 5B shows that the well-dispersed state of MWCNT fibers was maintained at the time of intratracheal instillation in the same manner as in the suspension before the instillation. A SEM image (Fig. 5C) reveals that MWCNT fibers were well-dispersed in the alveoli on Day 1 after instillation, and that the dispersed fibers appear to be penetrated from the cytoplasm of alveolar macrophages after phagocytosis or incomplete phagocytosis.

Metal impurities in the MWCNT

The qualitative analysis by LA-ICP-MS revealed that the MWCNT contained nickel, cobalt, iron, manganese and chromium. The AAS analysis showed that the contents of iron, chromium and nickel in MWCNT were 4,400, 48 and 17 ppm (wt/wt), respectively, and that the manganese and cobalt contents were below the quantitative detection limit of 6 ppm.

Discussion

Length and width of MWCNT fibers

The lengths and widths of MWCNT fibers measured in the present study are in good agreement with those reported by Takagi *et al*[1]. The fraction of fiber lengths exceeding 15 μm were apparently greater in the MWCNT used in the study of Poland *et al.*[3] than in the present study. Since we used the same MWCNT manufactured by Mitsui & Co. Ltd, there might be a difference in the measurement method of fiber length between the present study and that Poland *et al.*'study[3].

Indeed, we measured fibers greater than 0.5 μm in length on enlarged SEM photoprints. The present data of length and width of MWCNT fibers suggest that MWCNT might stimulate asbestos-like, length-dependent, pathogenic behavior in the lung. Poland *et al.*[3] demonstrated that long MWCNT fibers peritoneally injected in the abdominal cavity of female mice exert asbestos-like pathogenicity such as frustrated phagocytosis. Indeed, physical dimensions such as length and width of biopersistent fibers are known to be critically important determinants for the induction of mesotheliomas[15, 16]. Indeed, the length of asbestos fibers is known to be a critically important determinant for asbestos-induced carcinogenesis[14, 15]. *In vitro* exposure of cultured LLC-MK_2 cells to long crocidolite asbestos fibers was reported to induce formation of binucleated cells leading to polyploidy, resulting from sterical blocking of cytokinesis by long fibers[17]. Another key factor for pathogenic, asbestos-like behavior is whether MWCNT is persistent in the lung and pleural cavity in the same manner as in the case of asbestos fibers suggested by Donaldson and Tran[18]. Our separate paper[10] demonstrated that a single intratracheal instillation of well-dispersed MWCNT fibers in rats caused persistent deposition of MWCNT in the alveolar space and wall, in addition to a tendency of MWCNT deposition in the bronchus-associated lymphoid tissue to gradually increase after the instillation. Those results suggest that the MWCNT is persistent in the lung and pulmonary lymphatic vessels. Therefore, it can be inferred that different pulmonary toxicities might be manifested, depending on the length of MWCNT fibers and their biopersistence. A further study with long-term observation will be needed to examine the biopersistence of MWCNT in the lung and pleura.

Dispersion of MWCNT fibers in the suspension and in the lung tissue

Together with addition of Tween 80 into PBS, 5-min ultrasonication was found to facilitate good dispersion of the agglomerated MWCNT fibers in the suspension. Since Tween 80 was reported to increase the susceptibility to oxidative stress in rat thymocytes at 10–30 μg/ml under *in vitro* conditions[19], the concentration of Tween 80 used in the present study was fixed at a concentration of 0.1%, in order to keep good dispersion and to minimize the pulmonary toxic responses to Tween 80. In our intratracheal instillation study[10], MWCNT was suspended in the PBS containing 0.1% Tween 80 and ultrasonicated for 20 min with an ultrasonic homogenizer. Then, the ultrasonicated suspension of MWCNT was further subjected to additional ultrasonication for 30 s with a sonicator immediately before intratracheal instillation. This repeated ultrasonication was performed to maintain good dispersion of MWCNT in the suspension, because the MWCNT appeared visually to re-agglomerate when the suspension was left for about 30 min during the period for intratracheal instillation.

It is interesting to note in the present SEM observation (Fig. 5A) that some MWCNT fibers were overlapped and twisted with each other, but there were neither clumped nor aggregated particles. Besides, knot-like blocks at the ends of fibers were observed occasionally. Presumably, formation of the knot-like structure might be causally related to the presence of residual catalyst particles on which two or more filaments of MWCNTs grow during the gaseous CVD process. The simulation experiment (Fig. 1) revealed that the microspray cannula used in the present study would allow delivery of the MWCNT suspension as a mist-like colloid into the distal end of the trachea, as evidenced by the well-dispersed state of MWCNT fibers (Fig. 5B) similar to that seen in the suspension before the instillation. The SEM observation (Fig. 5C) evidenced that MWCNT fibers were well-dispersed in the alveoli on Day 1 after intratracheal instillation at a dose of 160 μg/rat. Notably, cytoplasmic penetration of the dispersed MWCNT fibers after phagocytosis by alveolar macrophages (Fig. 5C) could be categorized as frustrated and incomplete phagocytosis, as proposed by Poland *et al.*[3] and Hubbs *et al.*[20], respectively. Therefore, it can be concluded from both DLS measurement and SEM observation that the MWCNT fibers were well-dispersed in the suspension by 20-min ultrasonication in the presence of 0.1% Tween 80 as a dispersant.

Dispersion or agglomeration of SWCNT administered by intratracheal instillation and pharyngeal aspiration has been reported to affect the pulmonary toxic responses. Warheit *et al.*[6] reported that intratracheal instillation of SWCNT produced mortality due to suffocation in 15% of the dosed rats, resulting from mechanical blockage of the respiratory tract by SWCNT which was highly electrostatic and did not disperse into single fibers. Mercer *et al.*[5] reported that pharyngeal aspiration of well-dispersed SWCNT in mice induced a potent interstitial fibrotic reaction with wide distribution of the dispersed fibers into the alveolar interstitium in the absence of granuloma formation. On the other hand, Shvedova *et al.*[9] showed that pharyngeal aspiration of agglomerated, less-dispersed SWCNT in mice induced granulomatous lesions associated with hypertrophied epithelial cells surrounding SWCNT aggregate as well as inflammation and interstitial fibrosis. Taken together, it can be inferred that good dispersion of MWCNT fibers in the suspension and ultimately in the lung tissue might be involved in the rat pulmonary lesions induced by intra-

tracheal instillation of MWCNT pretreated in the same manner[10].

Metal impurities

MWCNT iron, chromium and nickel contents were 4,400, 48 and 17 ppm (wt/wt) in the present study. They were estimated to be equivalent to lung burden of 0.7, 0.0077 and 0.0027 μg/rat, respectively, when 160 μg of MWCNT were intratracheally instilled. We examined the pulmonary toxic responses to these metal impurities on the basis of a literature survey. Toya *et al.*[21, 22] reported that intratracheal instillation of chromium and nickel fumes in rats induced a slight degree of pulmonary lesions at doses of 3.4 mg Cr/kg and 1.4 mg Ni/kg body weight. It can be inferred on the basis of these reported findings that the administration of 0.0077 and 0.0027 μg/rat, which are equivalent to 0.031 and 0.011 μg/kg body weight, would not induce any pulmonary lesions. Shvedova *et al.*[7] reported that *in vitro* exposure of human keratinocyte cells to unrefined SWCNT containing 30% iron produced cellular toxicity and oxidative stress as detected by the electron spin resonance (ESR) of the iron. Kagan *et al.*[8] reported that iron-rich SWCNT (26% Fe) caused significant loss of intracellular low molecular thiols (GSH) and accumulation of lipid hydroxides in both zymosan- and PMA-stimulated RAW 264.7 macrophages as compared with iron-stripped SWCNT (0.23% Fe). Shvedova *et al.*[9] also showed that pharyngeal aspiration of the purified SWCNT containing 0.23% iron in mice at a dose of 40 μg/head (equivalent to 0.09 μg iron) did not generate detectable signals from iron paramagnetic centers readily detectable by ESR spectroscopy in the unrefined SWCNT. Assuming similar distribution of 0.7 μg iron in the present study and 0.09 μg iron in the study of Shvedova *et al.* over the alveolar epithelial surface area, and normalizing to the equivalent alveolar epithelial surface area in rats (0.392 m^2/lung) and mice (0.068 m^2/lung) from a published morphometric analysis[21], the amount of iron in the unit alveolar epithelial surface area would be 1.7 $\mu g/m^2$ for rats and 1.3 $\mu g/m^2$ for mice. Taking the results of the *in vitro* and *in vivo* studies by Shvedova *et al.* and Kagan *et al.*[7–9] into consideration, any pulmonary responses to 4,400 ppm iron in the MWCNT might not contribute significantly to the observed pulmonary responses to 160 μg MWCNT. It can be concluded that 4,400 ppm (wt/wt) iron, 48 ppm chromium and 17 ppm nickel in the MWCNT were below the levels that would elicit positive pulmonary toxic responses to these metals.

Conclusion

Length and width of MWCNT fibers, dispersion of MWCNT in the PBS suspension containing 0.1% Tween 80 after ultrasonication, and metal impurities of MWCNT were investigated for an intratracheal instillation study. Mean length and width of single MWCNT fibers as measured on SEM photographs were 5.0 μm and 88 nm, respectively, and the fibers longer than 5.0 μm were 38.9% of all fibers measured. Both DLS measurement and SEM observation revealed that the MWCNT was well-dispersed in the suspension, at the time of intratracheal instillation, and in the alveoli on Day 1 after instillation. The AAS analysis showed that MWCNT iron, chromium and nickel contents were 4,400, 48 and 17 ppm (wt/wt), respectively, all of which were below the levels that would elicit positive pulmonary toxic responses to these metals.

Acknowledgements

The present study was financially supported by a Grant-in-Aid for Scientific Research from the Ministry of Health, Labour and Welfare of Japan. The authors are deeply indebted to Dr. Haruhiko Sakurai, Professor Emeritus of Keio University, for his fruitful discussion in the present study and to Dr. Makoto Ohnishi of the JBRC for his excellent assistance with metal analysis in MWCNT.

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