Cytotoxicity Study of High Temperature Wool (HT Wool) by Cell Magnetometric Evaluation

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Abstract: Cytotoxicity Study of High Temperature Wool (HT Wool) by Cell Magnetometric Evaluation: Yuichiro Kudo, et al. Department of Preventive Medicine and Public Health, Kitasato University School of Medicine—Objectives: We performed a cytotoxicity study by cell magnetometry, measured lactate dehydrogenase (LDH) activity by enzyme assay, detected DNA ladder formation, and performed morphological examination by electron microscopy in order to evaluate the safety of high temperature wool (HT wool), an asbestos substitute, using long and short chrysotile fibers (CF) as positive controls and phosphate buffered saline (PBS) as a negative control.

Methods: Alveolar macrophages were isolated from male Fisher rats. Following the addition of iron oxide particles (Fe_3O_4) to macrophages, HT wool, long or short CF was added. Then, the remanence strength was measured for 20 min after magnetization by an external field. Percent LDH release was calculated after determining LDH activity. DNA was detected using an apoptosis detection kit. Morphological observation was performed by taking electron micrographs of macrophages in the groups treated with HT wool and long- and short-CF. **Results:** Rapid relaxation, an indicator of decay of cytotoxicity, was observed by cell magnetometry immediately after magnetization was ended in the groups treated with HT wool and PBS, showing that HT wool causes no harmful effect on the cytoskeleton. The CF-treated groups had higher LDH activity than the PBS- and HT wool-treated groups. No fragmentation of DNA was observed in any group. In morphological observation, cytotoxicity in macrophages was lower in the HT wool-treated groups than in the CF-treated groups. **Conclusions:** The results suggest that HT wool has no cytotoxicity, as evaluated by cell magnetometry, enzyme assay, DNA ladder detection

and morphological examination.
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Key words: Cell magnetometry, Cytoskeleton, Cytotoxicity, High temperature wool (HT wool), Relaxation

It has been reported that asbestos causes pulmonary fibrosis and malignant tumors, and many *in vitro* and *in vivo* studies have shown its toxicity. Therefore, the use of asbestos has been restricted worldwide, including in Japan^{1–3}, and many asbestos substitutes have been developed. A variety of man-made vitreous fibers (MMVF) such as rock wool (RW) and glass wool are currently used as asbestos substitutes. Recently, a new type of RW, high-alumina, low-silica stone wool, high-temperature wool (HT wool), has been developed. Its chemical composition is characterized by a high concentration of Al_2O_3 and a low concentration of SiO_2 , low biopersistence, and a high melting point. These characteristics of HT wool have enabled its use to be broadened to heat insulation and other applications, and it has extensively replaced traditional types of RW. However, since HT wool has only been used for a short time, and the IARC categorizes it as unclassifiable, evaluation of its safety is urgently needed. Since most chemical substances enter the body through the airways and are processed by alveolar macrophages, evaluation of the effects on these cells should be a useful system for screening the physiological effects of chemical substances. Furthermore, alveolar macrophages, due to their phagocytic activity, are suited for the evaluation of insoluble particles such as dust, fibrous substances like asbestos and asbestos substitutes.

Cell magnetometry is an application of lung magnetometry, which was first reported by Cohen in 1973⁴. The principle of cell magnetometry is to induce uptake of iron oxide particles (magnetic particles) by phagocytes and to magnetize these particles by applying an external magnetic field, followed by measurement of the strength of the remanence after cessation of external

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Fig. 1. A. Scanning electron micrograph of HT wool fibers. Mean length, $13.34 \pm 12.92 \mu\text{m}$; mean width, $1.92 \pm 1.14 \mu\text{m}$. B. Scanning electron micrograph of long CF. Mean length, $2.6 \pm 2.3 \mu\text{m}$; mean width, $0.15 \pm 1.80 \mu\text{m}$. C. Scanning electron micrograph of short CF. Mean length, $1.17 \pm 1.74 \mu\text{m}$; mean width, $0.056 \pm 0.035 \mu\text{m}$.

magnetization, to evaluate the time course of changes in the magnetic field. The decay of the remanence after cessation of magnetization is called relaxation. During external magnetization, magnetic particles become aligned with the external magnetic field, and after its removal, these particles start to deviate from this alignment due to random rotation associated with intracytoplasmic translocation of phagocytic vesicles, and the time course of this change is called relaxation.

In the present study, the cytotoxicity of HT wool was evaluated by cell magnetometry, enzyme assay, DNA ladder detection and morphological examination by electron microscopy, using alveolar macrophages, which play a key role in the defense mechanism against foreign substances invading via the transairway route.

Materials and Methods

This experiment was performed in accordance with the Ethical Guidelines for Animal Experimentation adopted by the Institutional Review Board of Kitasato University School of Medicine (Approval No. 2004022).

Materials

Iron oxide particles, an indicator for cell magnetometry, were supplied by Toda Kogyo Co., Ltd., Japan, and HT wool, a test sample for evaluation of cytotoxicity manufactured by R Co., Ltd., Japan, was provided by the Rock Wool Association, Japan. The geometric mean diameter of the iron oxide particles was $0.26 \mu\text{m}$, and the geometric mean length and width (geometric standard deviation) of HT wool were 13.34 (12.92) μm and 1.92 (1.14) μm , respectively (Fig. 1A). As analyzed by

fluorescent X-ray diffraction, the chemical composition of HT wool was approximately 43% SiO_2 , 18% Al_2O_3 , 16% CaO , 10% MgO , 6% Fe_2O_3 , 2% TiO_2 , 2% Na_2O , 1% K_2O and 1% S. As positive controls, chrysotile fibers (CF) of two different lengths (long CF supplied by Japan Fibrous Materials Research Association, and short CF supplied by the Japan Association for Working Environment Measurement) were used (Fig. 1B&C). The fiber length and width of long CF were $2.6 \pm 2.3 \mu\text{m}$ (range: 0.2 – $100 \mu\text{m}$) and $0.2 \pm 1.8 \mu\text{m}$ (range: 0.04 – $1.00 \mu\text{m}$), respectively. Its chemical composition was approximately 46% MgO , 46% SiO_2 , 3% Fe_2O_3 , 2% Al_2O_3 , and 1% CO_2 . The fiber length and width of short CF was $1.2 \pm 1.7 \mu\text{m}$ (mostly below $5 \mu\text{m}$) and $0.056 \pm 0.035 \mu\text{m}$ (range: 0.02 – $0.03 \mu\text{m}$), respectively. Its chemical composition was approximately 42% MgO , 35% SiO_2 , 5% Fe_2O_3 and 1% FeO .

Alveolar Macrophage Preparation

Six male Fisher rats (F344/N Slc), weighing 200–250 g, were used. They were anesthetized with an intraperitoneal injection of sodium pentobarbital (100 mg/kg) and sacrificed by exsanguination from the abdominal aorta after making a midline abdominal incision. The lungs were collapsed by incision of the diaphragm. With the trachea exposed, a silicone tube was inserted through its opening and fixed. Alveolar lavage was carried out by infusing 4 ml of millipore filter filtration-sterilized 0.1% ethylenediamine tetraacetic acid in cold phosphate buffered saline (PBS), pH 7.4, into the lungs under gentle massage of the chest, followed by recovery of the lavage fluid. This procedure was performed 10 times in total,

and the alveolar macrophage-containing lavage fluid obtained was pooled and then subjected to centrifugation at 1,800 rpm for 10 min and suspended by the addition of Macrophage-SFM liquid (Life Technologies Inc., Rockville, MD, USA) to the pellet. A portion of the suspension was used for determination of cell counts by a hemocytometer after trypan blue staining.

The alveolar macrophage suspension prepared by this procedure was diluted with Macrophage-SFM liquid so that a given amount of aliquot gave 1×10^6 cells per well. Each aliquot was poured into a well (Nunc Co., Roskilde, Denmark), with a cell disc of 1-cm diameter, followed by incubation at 37°C in 5% CO₂ for 18 h as follows. In cell magnetometry, enzyme assay and DNA ladder detection, iron oxide particles suspended in PBS were added at a final concentration of 50 µg/ml to each well, followed by the addition of HT wool fibers suspended in PBS at a final concentration of 50, 100 and 150 µg/ml in the experimental groups (50, 100, 150 µg/ml HT wool-treated groups). Either long or short CF was added at a final concentration of 50 µg/ml in the positive control groups (long or short CF-treated group), and 50 µl PBS was added in the negative control group (PBS-treated group). For electronmicroscopic observation, iron oxide particles were not added, and instead, HT wool fibers at a final concentration of 150 µg/ml per well were added in the experimental groups, and either long or short CF (at a final concentration of 50 µg/ml) or 50 µl PBS was added, as positive and negative control groups, respectively.

Cell Magnetometry

Cell magnetometry was performed in the same manner as reported by Keira *et al.*⁵⁾ After culturing alveolar macrophages for 18 h as described above, the glass disc harboring adherent alveolar macrophages was taken out of each well and placed in a glass weighing bottle containing 1 ml Macrophage-SFM liquid. The specimens were subjected to external magnetization for 1/100 s under the magnetic field of a cell magnetometer apparatus. Immediately after that, recording for 20 min of the remanence strength, as measured with a fluxgate-type magnetometer, was started using a pen recorder. The apparatus was operated in such a way that the sample table passed over the probe once every 6 s. The temperature was maintained at 37°C by means of a hot-air fan with a thermostat placed beneath the magnetic shield. The curve, which shows the level of relaxation, was obtained by measuring the remanence strength for 20 min post-magnetization. Measurement of the remanence strength for 2 min post-magnetization gave a nearly linear curve after logarithmic transformation, and the point at which this curve intersected the y-axis was designated B_0 . When expressing the remanence immediately after magnetization as B_0 and the decay

constant as λ , the remanence at time t (in seconds) after termination of the external magnetic field can be represented by the formula $B=B_0e^{-\lambda t}$, and the decay constant (λ) was calculated based on this formula.

Enzyme Assay

After completing 18 h incubation, using the same method for the magnetometry, the culture media were harvested and centrifuged at 1,400 rpm for 10 min to collect the supernatant. Lactate dehydrogenase (LDH) activity in the supernatant was measured by the same method reported by Wroblewski *et al.*⁶⁾ using an LDH-UV Test Wako (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and recorded on a model U-3210 self-recording spectrophotometer (Hitachi Ltd., Tokyo, Japan). Total LDH activity was measured in the same manner after cytolysis with the addition of 40% Triton X-100. Percent LDH release was calculated according to the following formula after determining each LDH activity released from macrophages caused by the addition of HT wool (LDH activity released) and total LDH activity: Percent LDH release=LDH activity released $\times$ 100/total LDH activity.

DNA Ladder Detection

After completing 18 h incubation, using the same method for the magnetometry, DNA was extracted from the alveolar macrophages using an apoptosis ladder detection kit (Wako). The specimen thus prepared was subjected to DNA agarose gel electrophoresis. The agarose gel was prepared to contain agarose at a concentration of 1.5%. The DNA specimen (12 µl) and DNA molecular weight marker (500 bp marker: Wako) were loaded in a well with agarose gel, followed by electrophoresis at 100 V in Mupid-3 (Cosmo Bio Co., Ltd., Tokyo, Japan). After completion of electrophoresis, DNA was stained with ethidium bromide, followed by photography through a filter (254 nm) of a transilluminator (NLMS-20E type).

Morphological Observation

Scanning electron microscope observation was performed following culture of cells for 18 h, as described above. Macrophages adsorbed onto the polycationics-treated glass were washed with 0.1 M cacodylate buffer (pH 7.4). Then, following prefixation with 1% glutaraldehyde at 4°C for 3 h and washing, cells were fixed with osmium tetroxide at 4°C for 3 h and washed with cacodylate buffer. For transmission electron microscopic observation, specimens thus processed were further subjected to staining, dehydration, resin treatment, embedding, ultrathin slicing with an ultramicrotome, and electron staining, followed by evaluation with a model H-600 (Hitachi). For scanning electron microscope observation, specimens were subjected to conductive

Fig. 2. Relaxation curve. There was a statistically significant difference between the 50 and 100 $\mu\text{g/ml}$ HT wool-treated groups and short CF-treated groups (*: $p < 0.05$).

staining, dehydration, drying, and conductive treatment, followed by evaluation with a model S-4500 FE type (Hitachi).

Statistical Analysis

The results are shown as mean $\pm$ standard error of measurements obtained for 6 rats in both the control and experimental groups. All data were analyzed by one-way analysis of variance by Stat View 5.0, and statistical analysis with multiple comparisons was performed by Scheffe's test.

Results

Cell Magnetometry

Relaxation curves were constructed by plotting values of the remanence for 20 min post-magnetization (Fig. 2). The remanence strength showed rapid relaxation: $38.55 \pm 6.40\%$ in the PBS-treated group and 30.95 ± 2.85 , 30.55 ± 3.60 , and $39.41 \pm 6.10\%$ in the 50, 100 and 150 $\mu\text{g/ml}$ HT wool-treated groups, respectively. In the CF-treated groups, however, relaxation was delayed as compared with that in the PBS- and HT wool-treated groups, as shown by values of 51.80 ± 6.01 and $53.90 \pm 3.88\%$ in the long and short CF-treated groups, respectively (Fig. 2). Results of multiple comparisons by Scheffe's test showed no significant difference between the PBS- and HT wool-treated groups, whereas differences between the HT wool-treated and the long and short CF-treated groups

were statistically significant ($p < 0.05$) (Fig. 2). At 2 min postmagnetization, the decay constant values were smaller in the long and short CF-treated groups than in the PBS- and HT wool-treated groups: 3.25 ± 0.34 ($\times 10^{-3}/\text{s}$) in the PBS-treated group, 3.06 ± 0.29 ($\times 10^{-3}/\text{s}$), 2.97 ± 0.26 ($\times 10^{-3}/\text{s}$) and 2.93 ± 0.42 ($\times 10^{-3}/\text{s}$) in the 50, 100 and 150 $\mu\text{g/ml}$ HT wool-treated groups, respectively, versus 1.80 ± 0.30 ($\times 10^{-3}/\text{s}$) and 1.54 ± 0.29 ($\times 10^{-3}/\text{s}$), respectively, in the long and short CF-treated groups. Multiple comparisons by Scheffe's test showed no statistical significance in the difference between the PBS- and HT wool-treated groups. However, the differences between the HT wool-treated and both the long and short CF-treated groups were statistically significant ($p < 0.05$) (Fig. 3).

Enzyme Assay

The percent release of LDH activity was $18.00 \pm 2.88\%$ in the PBS-treated group and 21.82 ± 6.15 , 23.45 ± 4.73 , and $23.24 \pm 7.85\%$ in the 50, 100 and 150 $\mu\text{g/ml}$ HT wool-treated groups, respectively, and 33.68 ± 7.57 and $34.19 \pm 4.32\%$ in the long and short CF-treated groups, respectively. Multiple comparisons by Scheffe's test demonstrated statistically significant differences between the HT wool-treated and the long and short CF-treated groups ($p < 0.05$), with no significant difference between the PBS- and HT wool-treated groups (Fig. 4).

Fig. 3. Decay constant 2 min postmagnetization. There was a statistically significant difference between the 50 $\mu\text{g/ml}$ HT wool-treated groups and short CF-treated groups (*: $p<0.05$).

Fig. 4. Percent LDH release from alveolar macrophages. There was a statistically significant difference between the HT wool- and long and short CF-treated groups (*: $p<0.05$).

DNA Ladder Detection

No fragmentation of DNA was observed in the PBS-, HT wool- and CF-treated groups (data not shown).

Morphological Observation

Morphological observation was carried out using a scanning electron microscope. In the PBS-treated groups, macrophages were round and the cell surface was entirely

covered with numerous microvilli projecting in all directions (data not shown). In the 150 $\mu\text{g/ml}$ HT wool-treated group, some cells were stretched in the direction of the fibers, while others adhered around the fibers due to the large fiber width, with fibers penetrating through the middle of cells (Fig. 5). With the addition of 50 $\mu\text{g/ml}$ long CF, on the other hand, fibers were seen protruding out of the cell surface, and macrophages with a stretched

Fig. 5. (A) Electron micrograph of macrophages in the HT wool-treated group (SEM). The width of HT wool fibers is large, resulting in incomplete phagocytosis by macrophages. (B) Electron micrograph of macrophages in the HT wool-treated group (TEM). Cross-section of a HT wool fiber is seen in the macrophage cytoplasm, but no apparent degeneration of the cytoplasm is seen.

Fig. 6. (A) Electron micrograph of macrophages in the long CF-treated group (SEM). Fibers are seen protruding from the cell surface, and macrophages appear to adhere to fibers with scarce presence of microvilli. (B) Electron micrograph of macrophages in the long CF-treated group (TEM). Microvilli on the cell surface are smoothed, with vacuole formation in the cytoplasm.

cell membrane appeared to adhere to the fibers. Large numbers of fragmented CF were seen on the surfaces of macrophages (Fig. 6). With the addition of 50 $\mu\text{g/ml}$ short CF, thin, short fibers clung to the macrophages (Fig. 7).

Transmission electron microscopy demonstrated phagocytic vacuoles, surrounded by a clear membrane structure, in the cytoplasm beneath the microvilli in macrophages in PBS. The nuclei were relatively large with irregular shapes, and smooth-shaped endoplasmic reticulum and lysosomes were seen in the cytoplasm. With the addition of 150 $\mu\text{g/ml}$ HT wool, cross sections of HT wool were seen in the cell walls of macrophages. However, no apparent degeneration of the cytoplasm was observed (Fig. 5). On the other hand, projections on the cell surfaces were smoothed in the 50 $\mu\text{g/ml}$ long CF-treated groups. Chrysotile fibers were broken into small pieces and taken into phagocytic vacuoles, forming vacuoles in the cells. Vacuolization was more evident in

the cells that had taken up a large volume of CF. Cells with a condensed nucleus were rarely observed (Fig. 6). Vacuolization of the cytoplasm was more evident with addition of 50 $\mu\text{g/ml}$ short CF than with 50 $\mu\text{g/ml}$ long CF. In addition, there was an increase in the number of cells with condensed nuclei, and the most severe damage to macrophages was caused by the addition of short CF (Fig. 7).

Discussion

We evaluated HT wool fibers for possible cytotoxicity using alveolar macrophages, which are known to play a primary role in *in vivo* defense mechanisms against particles inhaled from outside of the body⁷⁾, by applying our unique method of cell magnetometric evaluation. Since most chemical substances enter the body through the airway and are processed by alveolar macrophages, evaluation of the effects of these cells should be a useful system for screening the physiological effects of chemical

Fig. 7. (A) Electron micrograph of macrophages in the short CF-treated group (SEM). Macrophages appear to adhere to thin, small fibers with scarce presence of microvilli. (B) Electron micrograph of macrophages in the short CF-treated group (TEM). Vacuole formation in the cytoplasm is observed, with increased cells with a condensed nucleus.

substances. Furthermore, alveolar macrophages, due to their phagocytic activity, are suited for the evaluation of insoluble particles such as dust and fibrous substances like asbestos and asbestos substitutes. The harmful properties of the following chemical substances have been investigated by *in vitro* cell magnetometry: limestone (Keira *et al.*, 1996)⁸⁾, chrysotile (Keira *et al.*, 1998)⁵⁾, gallium arsenide (Okada *et al.*, 1999)⁹⁾, silicon carbide whiskers (Watanabe *et al.*, 2000)¹⁰⁾, titanium dioxide (Watanabe *et al.*, 2002)¹¹⁾, cadmium oxide (Niitsuya *et al.*, 2003)¹²⁾, cadmium chloride (Cho, 2000)¹³⁾, arsenic chloride (Okada *et al.*, 2001)¹⁴⁾, and photocopier toner (Furukawa *et al.*, 2002)¹⁵⁾. Evaluation of the cytotoxicity of silicon carbide whiskers by Watanabe *et al.* in 2000¹⁰⁾ showed higher sensitivity of cell magnetometry in the detection of cytotoxicity, whereas no abnormalities were detected by the enzyme assay method. Similarly, Watanabe *et al.* reported that, in cell magnetometric evaluation, titanium dioxide in particle form did not cause cytotoxicity, but in a filamentous form caused cytotoxicity.

In *in vivo* studies on relaxation, Aizawa *et al.* reported in 1994¹⁶⁾ a delay after intratracheal instillation of gallium arsenide in rabbits. Möller *et al.* reported in 1996¹⁷⁾ a delay in smokers, and Fukushima *et al.* reported in 1995¹⁸⁾ facilitation of relaxation by influenza virus infection in rats.

Brain *et al.* argued in 1988¹⁹⁾, based on findings obtained in rats, that phagocytosis of iron oxide particles with time took place not as a result of exocytosis of the iron oxide particles but as a result of their rotation associated with translocation of intracellular organelles. Materials are incorporated in phagocytic vesicles after phagocytosis by macrophages, followed by their

digestion. Intracytoplasmic translocation of these phagocytic vesicles and the cytoskeleton (mainly microtubules) is thought to play an important role in their polymerization and depolymerization. The cytoskeleton, which is mainly composed of microtubules, microfilaments and intermediate-diameter filaments, is known to play an important role in the maintenance of the cytoplasmic structure, intracellular transport and cellular polarity^{20–23)}. It is thought that exposure to harmful substances causes delayed relaxation through their physical and chemical effects on the cytoskeleton^{4, 19, 24)}. Thus, delayed relaxation reflects a reduction of cytoskeletal function with rotation of phagosomes, and it is therefore considered to be an indicator of cytotoxicity. In cytotoxicity testing of silicon carbide whiskers, as noted above, no abnormality was observed when an enzymatic method for measurement of cell membrane permeability was used, but cellular damage was observed as delayed relaxation by cell magnetometry, showing its higher sensitivity in toxicity evaluation. Therefore, cell magnetometry appears to be valuable for the evaluation of cytotoxicity, in addition to conventional methods. The present study employed cell magnetometry, measurement of extracellularly leaked enzymes to evaluate damage to cellular membranes, DNA ladder detection to evaluate possible apoptosis as a process of cell death, and electronmicroscopic observation to evaluate morphological changes of subcellular structure. These methods enabled more comprehensive evaluation of cytotoxic effects on macrophages and could be useful for safety screening of chemical substances.

Cytotoxicity of fibers is considered to be determined by their chemical composition and physical properties (fiber size and fiber number). In the present study, relaxation was rapid in the HT wool-treated groups,

whereas it was delayed in the CF-treated groups. In this study, cytotoxicity was higher with short CF than with long CF. This appeared to result from the fact that the number of short CF per unit weight was greater than that of long CF, since the total surface area was larger with short CF at the same weight concentration. Furthermore, it has been reported that active oxygen is produced in macrophages by addition of asbestos. It is known that active oxygen, produced by alveolar macrophages, damages the lungs²⁶⁾, and thus, lung damage by asbestos could possibly be caused by active oxygen produced by macrophages. Relaxation was delayed more in the CF-treated groups than in the HT wool-treated groups. Furthermore, HT wool is characterized by a higher dissolution rate at pH 4.5 and a lower dissolution rate at pH 7.4 than that of traditional types of RW^{27, 28)}. The pH of macrophage phagosomes is 4.5. In other words, since HT wool was dissolved in phagosomes, its cytotoxicity in macrophages was lower than that of CF^{27, 28)}. The *in vitro* dissolution rate of silicon of HT wool was 59 ng/cm²/h at 37°C in Gambles solution (pH 7.5)²⁹⁾, while that of CF was 0.08 ng/cm²/h under the same conditions³⁰⁾. This shows that the dissolution rate of HT wool is higher than that of CF, and that the cytotoxicity of HT wool is lower than that of CF.

In the present study, we measured LDH as a biochemical indicator. Measurement of LDH leaked from the cytoplasm of alveolar macrophages has been utilized as an indicator of cytotoxicity⁶⁾. Our study results showed that the CF-treated groups had higher LDH activity than the PBS- and HT wool-treated groups. As mentioned above, since HT wool was dissolved in phagosomes, LDH release was lower in the HT wool-treated groups than in the CF-treated groups, suggesting HT wool fibers have lower cytotoxicity than CF^{27, 28)}.

DNA fragmentation, as determined by DNA ladder detection, is thought to be specific to apoptosis. In the present study, however, no fragmentation of DNA was observed in either the PBS- or HT wool-treated groups. On the other hand, Hamilton *et al.*³¹⁾ reported that chrysotile-exposed cells (human alveolar macrophages) showed formation of DNA ladders, which is characteristic of apoptosis, in 48-h cell cultures. The apparent difference between their results and those of our present study could be due to differences in the cell species and culture time.

Morphological examination by scanning microscopy and electronmicroscopy showed that cytotoxicity in macrophages was lower in the HT wool-treated groups than in the CF-treated groups. These results suggest that cytotoxicity after short-term exposure *in vitro* is less marked with HT wool fibers than with CF. We should further evaluate the safety of HT wool by lung magnetometry and morphological observation of the lung in *in vivo* inhalation studies.

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