Research on the safety of new chemicals such as “Nano materials” etc.

Title of Research:

17_PT02-01

Establishment of mechanism based assay protocol for hazard and carcinogenic risk of carbon based nanomaterials

Principal Investigator:

Hiroyuki Tsuda, MD, PhD (Professor, Nagoya City University, Nanotoxicology Project Lab)
3-1, Tanabedohri, Mizuho-ku, Nagoya, 467-8603, Japan
(tel)+81-52-836-3496, (e-mail) htsuda@phar.nagoya-cu.ac.jp

Summary of Research:

To evaluate the toxicity and carcinogenicity of carbon nanotubes (CNT), we are establishing an inexpensive intratracheal intrapulmonary spraying (TIPS) method for administration of CNTs via the airway. TIPS will greatly expand research into the mechanism of CNT-mediated tissue injury and carcinogenesis beyond what is currently possible using conventional expensive inhalation exposure testing. Work in our laboratory has shown that among multi-walled carbon nanotubes (MWCNT), MWCNT-7 (M-H company, 40 wall layers) and MWCNT-N (N company, about 30-50 layers), which form needle-like aggregates, are carcinogenic to the lung and pleura of rats. More recently, work in our laboratory and by others using TIPS have shown that MWCNT-A (Company C, 150 layers) and MWCNT-B with fewer layers (Company C, 15 layers) are also lung carcinogens. In the current study, double-walled carbon nanotubes (DWCNT) (diameter 1-3 nm) (Company A) were compared with the carcinogenic CNT, MWCNT-7, using the TIPS method: acute / subchronic / chronic toxicity / carcinogenicity was analyzed.

Methods: 10-week-old male rats were administered a total dose of 0.25 and 0.5 mg DWCNT and MWCNT-7 per rat for the short-term test, and 10-week-old male rats were administered a total dose of 0.125, 0.25, and 0.5 mg DWCNT or 0.5 mg for MWCNT-7 per rat for the long-term test. CNTs were suspended in a solvent (saline + 0.5% dispersant PF68PF copolymer) and administered every other day over the course of 15 days (total of 8 TIPS administrations). The control groups were untreated rats and rats administered the solvent. MWCNT-7, which is known to be carcinogenic in the lung and pleural mesothelium, was used as a positive control.

Short-term test: 3 and 8 weeks after beginning administration (1 and 6 weeks after the final TIPS administration): The distribution and shape of the administered CNTs was examined. Histopathological analysis of lung tissue was performed to determine lung tissue pathology. Tracheal and lung lavage fluid, pleural lavage fluid supernatant, inflammatory markers in the cell pellets, and biochemical material prepared from lung tissue were analyzed to determine the degree of inflammation. Inflammatory cytokines, DNA damage, and oxidative stress markers revealed that DWCNT was less toxic than MWCNT-7.

Subchronic test (52W): The number of granules and alveolar Mφ of the encapsulated granulations in lung specimens was greater in the DWCNT treated rats compared to the MWCNT-7 treated rats. Notably, the number of PCNA-positive alveolar epithelial cells was less in the DWCNT treated rats compared to the MWCNT-7 treated rats. Many MWCNT-7 fibers were not encased in granulations and were often deposited in the alveoli. No neoplastic lesions were observed in either group.

Chronic toxicity. Carcinogenicity test (104w):

Lung tumor incidence (adenoma + adenocarcinoma) was significantly higher in the 0.5mg DWCNT group, 7/24, (29.2%) compared to vehicle group, 1/25, ($P < 0.048$). The incidence of pleural malignant mesothelioma was significantly higher in the MWCNT-7 group, 16/25, (64%) compared to the vehicle group, 0/25, ($P < 0.0001$).

Less acute toxicity and more pronounced granulation encapsulation in the lungs of rats administered DWCNT compared to rats administered MWCNT-7 suggests that malignant pleural mesothelioma developed relatively early in the MWCNT group, resulting in death before lung tumors were able to develop, however, this is a topic that requires further study. We are also studying carbon nanohorns and nanobrushes containing SWCNT structures using this same method (currently 46w).

Timeline:

March 1, 2019 – February 29, 2020

Topics:

Research on the safety of new chemicals such as “Nano materials” etc.

Poster presentation at JCIA LRI Annual Workshop 2019 “Establishment of mechanism based assay protocol for hazard and carcinogenic risk of carbon based nanomaterials” (Tokyo, August 30th, 2019)

Publications:

1. Abdelgied M., Elgazzar AM., Alexander D., Alexander W., Numano T., Iigo M., Naiki-Ito A., Takase H., Abdou KB., Hirose A., Taquahashi Y., Kanno J., Abdelhamid M., Tsuda H., Takahashi S. Pulmonary and pleural toxicity of potassium octatitanate fibers, rutile titanium dioxide nanoparticles, and MWCNT-7 in male Fischer 344 rats, Arch. Toxicol., 93(4): 909-920, 2019
2. Abdelgied M., Elgazzar AM., Alexander TW., Numano T., Iigou M., Naiki-Ito A., Takase H., Hirose A., Taquahashi Y., Kanno J., Abdelhamid M., Khaled AA., Takahashi S., Alexander BD, Tsuda H. Carcinogenic effect of potassium octatitnate (POT) fibers in the lung and pleura of male Fischer 344 rats after intrapulmonary administration, Particle and Fibre Toxicology, https://doi.org/10.1186/s12989-019-0316-2 2019