PS 1257 Exposures to Carbon Nanotubes and Asbestos Induce Related but Distinct Profiles of Toxicologic Lung Pathology

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Carbon nanotubes (CNTs) are rapidly emerging as occupational and environmental lung toxicants. The increasing manufacture and applications of CNTs has prompted concerns surrounding their potential to cause adverse lung effects in light of asbestos-like characteristics such as high aspect ratio and biopersistance. Because of their novelty, the long-term health outcomes of CNT exposures in humans are unknown. CNTs may become agitated into aerosols in occupational settings, posing an inhalational threat to those who work in places of CNT manufacture, distribution, and usage. However, there are limited studies or inconsistent findings regarding CNT toxicology or their toxicological similarities to asbestos. In this study, we developed mouse models of exposure using repeated, low-dose oropharyngeal aspirations of multi-wall CNTs or crocidolite asbestos. Histopathological analysis of lung sections showed that while granulomatous inflammation was similarly induced in both exposures, CNTs caused type II pneumocyte (T2P) hyperplasia, while asbestos caused mixed-cell bronchoalveolar hyperplasia. Both exposures caused increases of fibrotic collagen as shown in Masson's trichrome stains. Fluorescent immunohistochemistry for T2P-specific proSPC showed that T2P number was substantially increased specifically in CNT-exposed lungs. These observations are significant considering that T2P cells are known to become hyperplastic in response to alveolar epithelial injury. Co-staining for proSPC and IL-1β showed that while both exposures increased IL-β+ cells in lung tissue, CNT-induced IL-1β increases were largely specific to T2Ps. These results illustrate that CNT and asbestos exposures may induce related but reproducibly distinct profiles of toxicologic lung pathology, and that T2Ps may be sensitive to CNT-induced toxicity. This suggests that CNTs and asbestos may differ in their mechanisms of toxicity and resultant injury in the distal airway.

PS 1258 Single-Walled Carbon Nanotubes (SWCNTs) Induce Vasodilation in Isolated Rat Aortic Rings

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Single-walled carbon nanotubes (SWCNTs) are used in biological systems with impact in biomedicine in order to improve diagnostics and treatment of diseases. However, their effects upon the vascular system are not fully understood. Endothelium and smooth muscle cells (SMC) communicate through the release of vasoactive factors such as nitric oxide (NO) to maintain vascular tone. The aim of this study was to evaluate the effect of SWCNTs on vascular tone using an isolated rat aortic rings model. Aortic rings were exposed to SWCNTs (0.1, 1 and 10 μg/mL) both in presence and absence of endothelium. SWCNTs induced vasodilation in both conditions, indicating that this effect was not dependent on endothelium. Moreover, blockage with L-NG-Nitroarginine methyl ester (L-NAME) did not modify the observed effect suggest vasodilation was independent on NO production. Together, these results indicate that SWCNTs induce vasodilation in the macrovasculature, may be through a direct interaction with SMC rather than endothelium without involving the NO production. Further investigation is required to fully understand the mechanisms of action and mediators involved in the signaling pathway induced by SWCNTs on the vascular system components under specific biological conditions.

PS 1259 Role of Stem-Like Cells in Carbon Nanotube-Induced Pulmonary Fibrosis

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Carbon nanotubes (CNTs) have generated great interest commercially with their diverse applications however, the risk of their adverse health effects is not well understood. Studies have shown that CNTs can induce pulmonary fibrosis in animal models. Since fibrosis is associated with aberrant tissue repair and extracellular matrix (ECM) accumulation, identifying the cells that are responsible for the repair and ECM production is fundamental to the understanding of fibrosis mechanism. We hypothesize that CNTs induce fibroblast stem-like cells (FSCs) and that such induction is essential to the development of fibrosis. Fluorescence activated cell sorting was used to isolate FSCs from CNT-treated normal human lung fibroblasts (NHLFs).The expression of stem cell markers and fibrogenic markers was examined using western blotting, immunofluorescence staining and confocal microscopy. Our results demonstrated for the first time that CNTs can induce FSCs from NHLFs as evidenced by their side population (SP) property and expression of stem cell markers ABCG2 and ALDH1A1. These cells, isolated from SP-positive FSCs, showed a high expression of type I collagen and α-smooth muscle actin, which are key biomarkers of fibrosis, as compared to non-SP cells. The induction of FSCs by CNTs was redox-sensitive since inhibition of oxidative stress by antioxidants including *N*-acetyl cysteine and catalase effectively inhibited the FSC induction. Together, our results support the existence of FSCs induced by CNTs and their putative role in fibrogenesis. This novel finding provides a new insight into the mechanisms of fibrosis and may aid in the development of early detection biomarkers and treatment strategies for the disease. [Supported by NIH grants R01-HL095579 and R01-ES022968 and by NSF grants EPS-1003907 and CBET-1434503]

PS 1260 Phototoxicity of Ethyl Maltol in Hairless Mice

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Ethyl Maltol (CAS # 4940-11-8) is a widely used, aliphatic ketone fragrance material which imparts an intensely sweet, fruity, bread-like odor. It has a total systemic exposure of 0.04 mg/kg body weight (bw)/day. The UV spectrum for this material demonstrates absorption in the region of 290 – 700 nm, and therefore the potential to be photoactivated. Additionally, ethyl maltol resulted in positive responses for phototoxicity *in vitro* using the 3T3 NRU phototoxicity test. To establish a No Observed Effect Level (NOEL) *in vivo* under use conditions, the phototoxic potential of topically applied ethyl maltol was evaluated in female Crl:SKH1-hr hairless mice. Mice (6/group) were administered 1, 7 or 30% (w/v) ethyl maltol in methanol. The formulations were applied to the dorsum and sides of the mouse (application area = approximately 25 cm^2). Before ultraviolet radiation (UVR) exposure, all mice were lightly sedated. Irradiation took place approximately 1 hour after dosing from a 6.5 kw long-arc xenon water-cooled lamp with a glass filter to attenuate mid-range ultraviolet (UVB) and simulate mid-latitude summer sunlight. A UVR dose of approximately 0.5 Minimal Erythema Dose (MED) was delivered over a period of approximately 30 minutes. The mice were placed 1.2 meters from the UVR source at the time of exposure. The mice were observed for 72 hours for dermal and clinical signs. Treatment with up to 30% ethyl maltol followed by UVR exposure, did not result in erythema, edema or flaking. No adverse body weight changes or clinical observations occurred in the study. A single topical administration of ethyl maltol at concentrations up to 30% to female hairless mice, followed approximately 1 hour later by a single exposure to solar-simulated ultraviolet radiation, did not result in any skin reactions indicative of phototoxicity. Under the conditions of the study, the No-Observed-Effect-Level (NOEL) for phototoxicity of ethyl maltol in mice is greater than or equal to 30%, the highest dose tested.

PS 1261 Photosafety Screening on Benzophenones Using Photochemical and Dermal Cassette-Dosing Pharmacokinetic Data

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Phototoxic responses can be caused by administration of phototoxins, followed by exposure to sunlight. Previously, a photosafety screening strategy by combined use of photochemical and cassette-dosing pharmacokinetic (PK) analyses was proposed for predicting phototoxic risk of systemically-exposed drugs. The present study aimed to confirm applicability of the strategy on dermally-taken chemicals and reliability of outcomes obtained from the strategy. Photochemical properties of 6 benzophenones (BZPs), including benzophenone (BZ), ketoprofen (KT), oxybenzone (OX), sulisobenzone (SB), dioxybenzone (DO), and mexenone (MX), were characterized by UV spectral analysis and reactive oxygen species (ROS) assay. Cassette-dosing approach on dermally-taken BZPs was conducted to obtain PK parameters in rat skin. For a comparison purpose, a 3T3 neutral red uptake phototoxicity test (3T3 NRU PT) and an *in vivo* phototoxicity study in rats were also carried out. All the BZPs exhibited strong UVA/B absorption, and BZ, KT, OX and MX yielded ROS under exposure to simulated sunlight; however, ROS generation from SB and DO was negligible. The type and amount of ROS generated from BZPs were quite different even though they have similar chemical structure. The dermal PK parameters of KT indicated the highest dermal distribution of all BZPs tested. From the photochemical and PK data, the phototoxic risk of BZPs was ranked as

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