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Evaluation of the fate and pathological response in the lung and pleura of brake dust alone and in combination with added chrysotile compared to crocidolite asbestos following short-term inhalation exposure

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ABSTRACT

This study was designed to provide an understanding of the biokinetics and potential toxicology in the lung and pleura following inhalation of brake dust following short term exposure in rats. The deposition, translocation and pathological response of brake-dust derived from brake pads manufactured with chrysotile were evaluated in comparison to the amphibole, crocidolite asbestos. Rats were exposed by inhalation 6 h/day for 5 days to either brake-dust obtained by sanding of brake-drums manufactured with chrysotile, a mixture of chrysotile and the brake-dust or crocidolite asbestos. The chrysotile fibers were relatively biosoluble whereas the crocidolite asbestos fibers persisted through the life-time of the animal. This was reflected in the lung and the pleura where no significant pathological response was observed at any time point in the brake dust or chrysotile/brake dust exposure groups through 365 days post exposure. In contrast, crocidolite asbestos produced a rapid inflammatory response in the lung parenchyma and the pleura, inducing a significant increase in fibrotic response in both of these compartments. Crocidolite fibers were observed embedded in the diaphragm with activated mesothelial cells immediately after cessation of exposure. While no chrysotile fibers were found in the mediastinal lymph nodes, crocidolite fibers of up to 35 μm were observed. These results provide support that brake-dust derived from chrysotile containing brake drums would not initiate a pathological response in the lung or the pleural cavity following short term inhalation.

Introduction

The study is unique in that it has examined the pathological response and fiber distribution in the lung and in the pleural cavity of brake dust from chrysotile containing brake drums. In the interim results on the lung which were presented in Bernstein et al. (2014), the brake dust from chrysotile containing brake drums was shown to produce no pathological response in the lung through 91 days following short-term exposure in rats. The study also demonstrated the importance of amphibole asbestos exposure in comparison to chrysotile in the etiology of asbestos related lung disease. This study was continued through 365 days post exposure in order to assess the evolution of these findings and includes further results from the lung analyses and from the analysis of the pleural cavity from the study including assessment of the visceral and parietal pleural surfaces.

Chrysotile fibers were found to be effective since the 1900s in manufacturing brake materials with the ability to withstand heat and control speed. The surface of the brake drums often needed to be sanded to assure a proper fit. This study was designed to evaluate the hypothesis of whether brake dust from sanded chrysotile containing brake drums

Abbreviations: Crl: Wi(Han), Wistar rats, Specific Pathogen Free from Charles River Deutschland; SEM, scanning electron microscope; WHO, World Health Organization; MMMF, man made mineral fibers; VDI, Verein Deutscher Ingenieure (English, Association of German Engineers); GMD, Geometric mean diameter; GML, Geometric mean length; GSD, Geometric standard deviation; MMAD, Mass median aerodynamic diameter; %CT/FOV, percentage of the elastin and collagen per area of lung tissue; CM, Confocal microscopy; TGF-ß, Transforming growth factor (TGF)-β; bFGF, Basic fibroblast growth factor; PDGF, Platelet-derived growth factor; CTGF, connective tissue growth factor.

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will produce a pathological response following short term exposure in rats. Brake dust has not been previously evaluated in animal studies.

The techniques used in this study have been designed to sample the thin pleural surfaces with minimal alteration of the homeostatic balance and fiber location. Two independent methods were developed for examining the translocation of fibers to the pleural cavity and any associated inflammatory response following exposure to either brake dust with added chrysotile, brake dust alone or crocidolite asbestos. These methods included the in situ examination of the lungs and pleural space including the visceral and parietal pleural obtained from freeze substituted tissue in deep frozen rats and the examination of the diaphragm as a parietal pleural tissue.

In examining the visceral pleural environment, including the subpleural lung, the visceral pleural itself, and the pleural space, a non-invasive method for determining fiber location, size, inflammatory and fibrotic response was used on rats which were deep frozen immediately after killing. In addition, the visceral pleural wall thickness and the amount of collagen per field of view (fibrotic response) in the visceral pleura was quantified using confocal microscopy procedures.

The diaphragm was chosen as the parietal pleural tissue for examination because it can be quickly removed at necropsy with minimal alteration of the visceral lung surface and it has a high density of lymphatic stomata (Negrini et al., 1991; Negrini and Moriondo, 2013). A fixed area which included lymphatic drainage sites (stomata) on the diaphragmatic surface was selected for examination of possible inflammatory response using scanning electron microscopy (SEM) and for the presence of fibers.

Methods

The aerosol generation/exposure, in-life and pathology phases of this study were performed by the Fraunhofer Institute for Toxicology and Experimental Medicine (Hannover, Germany) in compliance with the Principles of Good Laboratory Practice (German Chemicals Act §19a, Appendix 1, July 02, 2008, Federal Law Gazette I, No. 28, p. 1146) and the German animal protection law (Tierschutzgesetz of May 18, 2006, German Federal Law Gazette I, page 1206, 1313). The fiber counting and sizing was performed by Gesellschaft für Schadstoffanalytik mbH (Ratingen, Germany). The confocal microscopy was performed by Rogers Imaging (Needham, Massachusetts, USA).

Brake dust

The brake dust was produced directly from chrysotile-containing friction products (automotive drum brake shoes) by the RJ LeeGroup Ltd. (Monroeville, PA, USA). The shoes were designed to fit the drum brakes of mid-1960's Chevrolet Impala model cars. The friction material was evaluated and found to contain approximately 30% (by area) chrysotile asbestos (analyzed in accordance with EPA 600/R-93/116). No amphibole asbestos minerals have been observed in any of the aerosol or lung samples from these brake shoes or in the added chrysotile used in this study.

The brake drums were ground using a commercial AMMCO arc grinder (Model 8000, S/N 24788) with a modified dust collection system. The arc grinder is a motorized sander that is swept across the surface of the brake shoe with the dust collected on an attached 8×10 inch quartz micro-fiber filter that was used in place of a collection bag. A Tisch high volume air sampler sampling pump (Tisch Environmental Inc., Ohio, USA) was used following the filter to provide uniform sampling suction over the course of the grinding operation. All brake dust preparation took place at the RJ LeeGroup facility in a room equipped with an Aramsco Comanche® HEPA ventilation unit (Model 55011) with a nominal flowrate of 1800 cfm (50 m^3/min). The brake dust was produced directly from asbestos-containing friction products (automotive drum brake shoes) by the RJ LeeGroup Ltd. (Monroeville, PA, USA) as described previously (Bernstein et al., 2014).

Chrysotile

The chrysotile fiber used in this study had the mineralogical grade of 5R04 according to the Canadian chrysotile asbestos classification (Cossette and Delvaux, 1979). The chrysotile grade 5R04 sample was chosen based upon an evaluation of which chrysotile grade was ordered or supplied for use in brake manufacturing in a random search of 67 formulations dating from 1964 to 1986. All of the grade 5R04 chrysotile in these brakes was supplied by Johns-Manville. The chrysotile sample used in this study was obtained directly from Mine Jeffery Canada (formerly the Johns-Manville Mine).

Crocidolite asbestos

The crocidolite asbestos sample used in this study was from the Voorspoed mine in South Africa was obtained from the National Institute of Occupational Health – NIOH, South Africa. This mine is located in Limpopo Province which at the time when mining took place was called Transvaal Province. The chemical compositions of chrysotile, a serpentine asbestos, and crocidolite, an amphibole asbestos, have been described previously (Shedd, 1985; Virta, 2002). A key difference with this crocidolite asbestos sample is that it was received as produced without subsequent grinding. The crocidolite asbestos used previously in animal studies has been largely either the Union for International Cancer Control (UICC) or US National Institute of Environmental Health Sciences (NIEHS) prepared crocidolite. Both of these samples were ground extensively more than 30 years ago using large scale industrial mills resulting in size distribution not typical of the commercial product (Bernstein et al., 2013).

Experimental design

The experimental design of the study has been illustrated in the flowchart in Fig. 1 of Bernstein et al., 2014. All end points were analyzed for each group with the exception that lung digestion was not performed in the control group on Days 1, 2 and 7 in order to limit animal use.

Animal exposure

Groups of laboratory rats (Groups 1, 2, 3 and 4) were exposed for 6 h per day for 5 days to:

Group 1: Filtered air (negative control group) (Total 65 animals).
Group 2: Brake dust powder mixed with chrysotile 5R04 (Total 100 animals).
Group 3: Brake dust powder (Total 100 animals).
Group 4: Crocidolite asbestos (Total 100 animals).

For groups 2 and 4, the exposure concentrations were set based upon the number of fibers longer than 20 μm/cm^3. In group 2, the chrysotile concentration was increased over that recommended by the EC Biopersistence Protocol (Bernstein and Riego-Sintes, 1999) of 100 fibers L > 20 μm/cm^3 due to the tendency of chrysotile to clump (this was minimized through the use of the cyclone, see below). Group 3 was included as a comparative exposure of the brake dust particulate material (with a relatively low aerosol concentration of chrysotile fibers) using a similar gravimetric exposure concentration as the brake dust component of group 2. A negative control group 1 was exposed in a similar fashion to filtered air.

Weanling (8–10 weeks old at exposure) male Wistar rats [Crl: WI(Han)], Specific Pathogen Free from Charles River Deutschland, Sulzfeld, Germany) were used. The rats were exposed by flow-past nose-only exposure for 6 h/day for a period of 5 consecutive days. In groups 2, 3, and 4; 7 animals per sub-group were allocated for lung burden evaluation at each time point. In the control group 1; 5 animals per sub-group were allocated for lung burden evaluation (no animals at days 1, 2 & 7). For the Confocal lung and histopathology, 3 animals per