Toxicology and Applied Pharmacology 334 (2017) 24–34

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/taap

Comparative health effects in mice of Libby amphibole asbestos and a fibrous amphibole from Arizona

Jean C. Pfau[a,*], Brenda Buck[b], Rodney V. Metcalf[b], Zoie Kaupish[a], Caleb Stair[a], Maria Rodriguez[a], Deborah E. Keil[a]

[a] Department of Microbiology and Immunology, Montana State University, Bozeman, MT 59717, USA
[b] Department of Geoscience, University of Nevada Las Vegas, Las Vegas, NV 89154, USA

ARTICLE INFO

Keywords:
Amphibole
Asbestos
Autoantibodies
Lung fibrosis
Pleural fibrosis

ABSTRACT

This project developed from studies demonstrating that Libby Amphibole Asbestos (LAA) causes a non-typical set of health outcomes not generally reported for asbestos, including systemic autoimmunity and an unusual and devastating lamellar pleural thickening that progresses to severe pulmonary dysfunction and death. Further, mineral fiber mixtures with some similarities to LAA have recently been discovered in southern Nevada and northwestern Arizona, where the material exists in extensive recreational areas and is present in yards, roads, parking lots and school yards. The objective was to compare the health outcomes in mice exposed to either LAA or the fibrous amphiboles collected in Arizona at the Lake Mead National Recreational Area at very low doses to represent environmental exposures. In this study, the fibrous amphibole asbestos sample from Arizona (AzA) is composed of winchite (69%), actinolite (22%), and non-amphibole minerals (9%) and has a mean aspect ratio of 16.7 ± 0.9. Fibrous amphibole asbestos from Libby (LAA) is composed of winchite (70%), richterite (9%), tremolite (5%), and non-amphibole minerals (16%) with a mean aspect ratio of 8.4 ± 0.7. C57BL/6 mice were exposed by oropharyngeal aspiration to fiber suspensions at a very low dose of 3 μg/mouse. After seven months, both LAA- and AzA-exposed mice had indices of chronic immune dysfunction related to a T_H17 cytokine profile, with B cell activation, autoantibody production and proteinuria, suggesting kidney involvement. In addition, both exposures led to significant lung and pleural fibrosis. These data suggest that there is risk of pulmonary disease and autoimmune outcomes with environmental exposure to amphibole asbestos, and that this is not limited to Libby, Montana.

1. Introduction

Multiple studies have chronicled the devastating health outcomes that resulted from asbestos exposure in Libby, Montana. While the rates of pulmonary fibrosis (asbestosis) and cancer (mesothelioma, pulmonary carcinoma) are significantly elevated among people exposed to the Libby amphibole asbestos (LAA), the predominant negative health outcomes include systemic autoimmunity and a progressive pleural fibrosis that may also be autoimmune in nature through production of mesothelial cell autoantibodies (MCAA) (Peipins et al., 2003; Pfau et al., 2005; Rohs et al., 2008; Sullivan, 2007; Szeinuk et al., 2016; U.S. Environmental Protection Agency, R, 2011; Whitehouse et al., 2008; Winters et al., 2012; Larson et al., 2010a; Gilmer et al., 2016; Hanson et al., 2016; Marchand et al., 2012; Serve et al., 2013). Using a wildtype mouse model (C57BL/6), we have corroborated these outcomes in mice, providing a critical tool for evaluation of the relative toxicity of other mineral fibers (Blake et al., 2008; Ferro et al., 2013; Gilmer et al., 2015; Pfau et al., 2013; Pfau et al., 2008; Zebedeo et al., 2014).

LAA exposures occurred due to contamination of vermiculite, which was mined outside of Libby for decades and used throughout the community in buildings, gardens, and playgrounds. This meant that there was a wide range of exposures, from high occupational exposures to relatively low, environmental exposures (Noonan, 2006; Noonan et al., 2015). Recently, the Environmental Protection Agency (EPA) conducted a risk assessment specifically for LAA based on a study that showed that significant negative health effects were occurring at extremely low exposure levels (Lockey et al., 2015). LAA is the first asbestiform fiber for which a toxicity value (Reference concentration, RfC) has been derived to help define a remediation target that reduces to acceptable levels the risk of acquiring non-malignant respiratory

Abbreviations: ANA, antinuclear autoantibodies; AzA, Arizona amphibole asbestos; EDS, energy dispersive spectroscopy; EPMA, electron probe microanalysis; LAA, Libby amphibole asbestos; LERP, Libby Epidemiology Research Program; MCAA, Mesothelial Cell Autoantibodies; RfC, Reference Concentration; SAED, selected area electron diffraction

* Corresponding author at: Department of Microbiology and Immunology, 960 Technology Blvd, Rm120, Bozeman, MT 59718, USA.
E-mail addresses: jean.pfau@montana.edu (J.C. Pfau), buckb@unlv.nevada.edu (B. Buck), metcalfr@unlv.nevada.edu (R.V. Metcalf), Deborah.keil@montana.edu (D.E. Keil).

http://dx.doi.org/10.1016/j.taap.2017.08.022
Received 14 June 2017; Received in revised form 26 July 2017; Accepted 31 August 2017
Available online 08 September 2017
0041-008X/

disease. The RfC_{LAA} was released on December 8, 2014 at 0.00009 PCM f/cm^3 (Phase Contrast Microscopy fibers/cm^3) (U.S. Environmental Protection Agency, R, 2015). The dramatic outcome of this fiber-specific health assessment, based on a specific non-cancer outcome (pleural fibrosis), emphasizes the need to evaluate health impacts based on variable mineral fiber composition from different sites. Similar to the study used by the EPA, the Libby Epidemiology Research Program (LERP, ATSDR, TS000099-01) has shown that 50% or more of people exposed to Libby Amphibole suffer from pleural scarring, and that this scarring can dramatically impact pulmonary function, eventually leading to significant disability and death (Szeinuk et al., 2016; Black et al., 2014). Amphibole mineral fibers are found in soils and rock outcroppings in many parts of the U.S. and around the world, leading to human exposures to naturally occurring asbestos (NOA) through numerous routes, including land development, recreation, and use of the material in roads, parking lots, and playgrounds (Abakay et al., 2016; Bayram & Bakan, 2014; Carbone et al., 2016; Cooper et al., 1979; Environmental Protection Agency, US, 2008; Paoletti et al., 2000; Van Gosen, 2007; Wylie & Candela, 2015). Thus, the public health impacts of on-going, current exposures, in addition to exposures over the last few decades, could be tremendous. Land use decisions in areas where NOA fibers are discovered need to be based on strong data that can be used in evaluating the application of the very low Libby RfC more broadly to other amphibole NOA.

Amphibole asbestos (fibrous amphiboles) has been reported in rocks, soils, dust, and air from areas in southern Nevada and northwestern Arizona on either side of the Colorado River near Hoover Dam (Buck et al., 2013; Metcalf & Buck, 2015; Tetra Tech, I, 2014). The amphibole asbestos material exists in extensive recreational areas and is present in yards, roads, parking lots and school yards in and around Boulder City, Nevada (Buck et al., 2013; Metcalf & Buck, 2015). These materials continue to be disturbed both by road construction/urban development but also by natural desert processes that produce dust storms throughout the year. Human exposures may already be extensive. The pathogenicity of this material is unknown, although environmental exposure to asbestos in southern Nevada is supported by a study showing atypical distribution of mesothelioma among women and young people (Baumann et al., 2013; Baumann et al., 2015). Amphibole asbestos in southern Nevada is predominantly the regulated mineral actinolite, while on the Arizona side the dominant asbestos mineral, referred to here as Arizona amphibole asbestos (AzA), is winchite, and is similar in composition and morphology to LAA. The relative pathogenicity may determine the need for a public health risk assessment for both cancer and non-cancer outcomes in this region. This study was designed to initiate this critical area of study, using our well-characterized mouse model.

2. Materials and methods

2.1. Amphibole minerals

LAA was provided by the EPA as a composite sample of asbestos-rich rock samples collected from multiple sites in the W.R. Grace mine outside of Libby, Montana. The LAA sample was previously characterized using a suite of methods including transmission electron microscopy (TEM) with selected area electron diffraction (SAED) (Duncan et al., 2014); scanning electron microscopy (SEM) with energy dispersive spectroscopy (EDS) (Gavett et al., 2016), x-ray diffraction (Lowers et al., 2012), and wavelength dispersive electron probe microanalysis (EPMA) (Meeker et al., 2003).

The fibrous amphiboles in this Arizona-Nevada region originated by hydrothermal alteration of granitic host rocks where asbestos minerals were precipitated as fracture-fill veins. In order to produce an AzA sample of high purity, a single sample of AzA in a centimeter-wide, asbestos-rich vein was collected from granitic rock of the Wilson Ridge pluton in the Lake Mead Recreation Area in northwestern Arizona. We used dental tools to separate AzA fibers in the vein from the rock matrix. This method decreases the amount of contaminant accessory minerals such as quartz, feldspar, and mica, which co-occur with the fibrous amphiboles. Scanning electron microscope-energy dispersive spectroscopy (SEM-EDS) analyses were performed on 470 particles to measure particle size and shape, and mineral chemistry. Wavelength dispersive electron probe microanalysis of a polished thin section of the AzA vein material provided quantitative chemical analyses that were used to classify the AzA minerals, methods comparable to that used by the USGS to classify LAA samples (Meeker et al., 2003). Characteristics of these fibers are summarized in Table 1. The fibers used in the current study are shown in columns 1 and 3 of Table 1.

Table 1
Fiber characteristics.

Characteristics	LAA (whole sample)[a,b]	AzA (amphibole only)	AzA (whole sample)[b]
Mineralogy	Winchite (70%), Richterite (9%), Tremolite (5%) Non-Amphibole (16%)	Winchite (76%), Actinolite (24%)	Winchite (69%), Actinolite (22%), Non-Amphibole (9%)
Morphology			
Analytical method	TEM	SEM	SEM
Min width (μm)	0.05	0.2	0.2
Max width (μm)	3.0	17.5	45.6
Mean width (μm)	0.36 ± 0.01	0.7 ± 0.1	1.3 ± 0.2
Min length (μm)	0.2	1.0	1.0
Max length (μm)	43.6	151.0	151.0
Mean length (μm)	2.3 ± 0.2	9.0 ± 0.7	9.7 ± 0.7
Min aspect ratio	1.0	1.0	1.0
Max aspect ratio	145.3	263.4	263.4
Mean aspect ratio	8.4 ± 0.7	18.2 ± 1.1	16.7 ± 0.9
Number of particles	510	427	470

[a] Data from (Duncan et al., 2014; Lowers et al., 2012; Meeker et al., 2003).
[b] Used in the current study.

All fibers were suspended in sterile phosphate buffered saline (PBS, pH 7.4), and sonicated (Branson Ultrasonics, Danbury, CT) for 5 min prior to use to minimize aggregation of the fibers.

Endotoxin testing of the fiber suspensions was performed using the PyroGene® Recombinant Factor C Endotoxin Detection System (Cambrex Bioscience, Walkersville, MD), following the manufacturer's protocol. Tested samples included the sterile PBS, suspended LAA (1.0 and 0.1 mg/ml) in sterile PBS, suspended AzA (1.0 and 0.1 mg·ml) in sterile PBS, plus *E. coli* O111·B4 (Sigma, St Louis, MO) lipopolysaccharide (LPS)-spiked samples, all against a standard curve provided with the kit. Briefly, all prepared samples and standards were placed in a 96-well plate at 100 μl/well, and incubated for 10 min at 37 °C. Detection working reagent was prepared and then added to all wells, and the plate was read immediately on a fluorescence plate reader (Fluorskan Ascent FL, ThermoFisher) at 380 excitation/440 emission. The plate was incubated for an hour at 37 °C, then read again with the same settings using the Ascent Software, to allow calculation of the change in fluorescence (ΔRFU), which was then plotted against the standard curve. Endotoxin was not detected in any of the samples, with a detection limit of 0.01 EU/ml.

2.2. Mice and exposures

All experiments with mice were approved by the Montana State University Institutional Animal Care and Use Committee (IACUC). The mice used were wild type C57BL/6 (Charles River, Seattle, WA) maintained in the Montana State University Animal Resource Center. These mice were housed under specific pathogen free (SPF) conditions