Potential Role of Toxicity Pathway Analysis in Understanding Multiple Modes of Action in Asbestos-Induced Adverse Respiratory Health Outcomes

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Abstract

Asbestos-containing materials may release asbestos fibers into the air during product use, demolition work, building or home maintenance, repair, and remodeling. Adverse health outcomes of asbestos are evident at the molecular level (DNA damage, lipid peroxidation, *etc.*) as well as at the tissue and whole organism level (fibrosis, mesothelioma, and lung cancer, *etc*). An overview of the literature was undertaken to identify and explore the potential modes of action of asbestos-induced disease, many of which involve reactive oxygen species (ROS). Asbestos-induced ROS production potentially from chronic inflammation, surface reactivity, *etc*, is associated with multiple modes of action in carcinogenicity (*e.g.*, genotoxicity, cytotoxicity). We examined evidence for multiple mechanisms of asbestos and how they may contribute to the overall response to asbestos exposure, and to understand how these mechanisms may lead to the resulting adverse health outcomes. This analysis also examined the response to specific fiber types as an aid to elucidating the key determinants of fiber toxicology. The effect of the mineral form is discussed in terms of type and relative magnitude of biological response. Overall, our analysis finds that chrysotile and amphibole asbestos may contribute to ROS production differentially both in terms of magnitude and potential mechanisms of response. However, these findings highlight the data gaps in determining how different fibers lead to variable downstream molecular events likely to result in asbestos-induced disease. These limitations in the available data need to be addressed to further understand key differences between fiber types that lead to varied health effects. Defining a toxicity signature profile for particular fiber types using new methodologies, particularly genomics and proteomics, would supply information on signaling pathways involved in response to asbestos exposure. Alternatively, these methodologies may also be used to define signature profiles for particular asbestos-induced disease endpoints. Information obtained from toxicity pathway based analyses for multiple fiber types, target tissues and adverse health endpoints will aid in defining determinants of toxicity of specific fiber types.

Introduction

- Asbestos is a known human carcinogen (IARC Group 1) whose mechanism of action is not completely known.
- The term 'asbestos' encompasses multiple mineral fiber types, including serpentine (chrysotile) and amphibole (crocidolite, tremolite, actinolite) family members. These family members differ in morphology as well as chemical composition, with the biological importance of these differences not completely elucidated.
- Asbestos exposure occurs through occupation (mining, auto repair) as well as through every day activities.
- Multiple modes of action are proposed following asbestos exposure, including reactive oxygen and nitrogen species production (ROS/RNS), chronic inflammation, genotoxicity, cytotoxicity and cell proliferation.
- Determinants of fiber toxicity play a role in different biological effects of asbestos, but detailed information on this for all fiber types is lacking.
- The pathogenesis of many asbestos-associated diseases is associated with a persistent inflammatory response, initiated directly or indirectly by ROS. Levels of ROS/RNS varies with the type of asbestos, although which characteristics of the fibers leads to this variable response is not yet known.
- Limited information is available for all fiber types on specific signaling pathway induction following exposure.
- New toxicity testing methodologies, particularly genomics and proteomics, could aid in answering many of the remaining questions on specifics of asbestos mode of action.

Figure 1. Transmission electron micrographs of two forms of asbestos: a. Chrysotile bundles and b. Amphibole fibrils. (Images obtained from USGS.)

Asbestos-Induced Health Effects

- Asbestos exposure may increase the risk of asbestosis and other nonmalignant lung and pleural disorders, including pleural plaques, pleural thickening, and pleural effusions.
- Asbestos has been classified as a known human carcinogen by the U.S. Department of Health and Human Services, the EPA, and the International Agency for Research on Cancer.
- In addition to lung cancer, mesothelioma and laryngeal cancer, some studies have suggested an association between asbestos exposure and gastrointestinal and colorectal cancers, as well as an elevated risk for cancers of the throat, kidney, esophagus, and gallbladder. However, the evidence is inconclusive. Asbestos exposure is also associated with autoimmune disease.

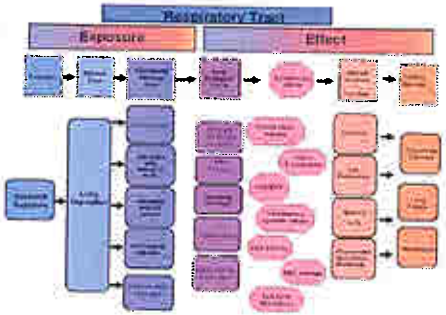

Figure 2. Asbestos exposure leads to disease through multiple mechanisms. Of interest is how these mechanisms overlap and interact to result in disease.

Determinants of Toxicity

Determinants of Fiber Toxicity: Proposed Impact on Biological Activity

Biological Activity	Length	Width	Mineralogy	Morphology	Density	Surface Area	Surface Composition
Inflammation	X	X	X		X	X	X
Cytotoxicity			X				X
Genotoxicity	X		X			X	X
Endocytosis	X	X		X			
Translocation	X	X	X	X	X		X
Dissolution	X	X	X				X

Table 1. Fiber dimension (width and length) and other characteristics such as chemical composition, surface area, solubility in physiological fluids, durability, surface charge, and surface reactivity may all play important roles in both the biologically significant fiber dose and fiber toxicity.

Multiple Modes of Action

Chronic Inflammation

Chronic inflammation from fiber exposure involves the prolonged release of ROS, cytokines and growth factors in the lungs or other target tissue. The unregulated or persistent release of these inflammatory mediators may lead to tissue injury, scarring by fibrosis and proliferation of epithelial and mesenchymal cells.

- Longer fibers (>15um) lead to an increased production of ROS due to 'frustrated phagocytosis', a term to describe the inability to fully phagocytose long fibers resulting in an increase in local neutrophil response leading to increased tissue damage.
- Small fibers (<5um) may remain in place or may be removed across the bronchiole or alveolar membrane and transported to the interstitial and pleural space through the blood vessels or lymphatic system.

Reactive Oxygen Species

- Asbestos exposure leads to increased ROS production through various signaling pathways.
- This increased production may be from direct fiber-cell interactions, respiratory burst and/or the chemical composition of fibers.
- Limited information is available on differences in types of ROS produced by specific fiber types.

Figure 3. Signaling pathways activated by ROS (Biocarta). These pathways are a sample of those activated following ROS production. The involvement of these pathways in multiple cellular responses adds to the variability in response to asbestos exposure.

Genotoxicity

Genotoxicity following exposure to asbestos fibers has been described as the result of two distinct mechanisms, described as direct and ROS/RNS-induced genotoxicity.

- **Direct genotoxicity:**
  - the physical interference of mitosis by fibers.
  - often results in chromosomal aberrations and aneuploidy due to interruption of mitosis.
  - longer fibers (>15um) are thought to play a major role in this form of genotoxicity.
- **ROS/RNS-induced genotoxicity:**
  - ROS/RNS production leading to DNA damage.
  - the amount of ROS/RNS produced following exposure to asbestos varies related to the chemical composition (iron) as well as other physical characteristics (solubility, size) that impact fiber clearance following exposure.
  - ROS/RNS production takes the form of hydroxyl radical as well as peroxynitrite, both of which are associated with 8-OHdG and single-strand base pair damage.

Cytotoxicity & Cellular Proliferation

The initial stages of any fibrotic and/or tumorigenic response involve cytotoxicity and compensatory cellular proliferation. Multiple signaling pathways may be involved leading to these endpoints (Figure 4). Asbestos exposure can lead to both increased and decreased cellular proliferation as observed in recent studies.

Figure 4. EGF signaling pathway (Biocarta). The epidermal growth factor (EGF) peptide induces cellular proliferation through the EGF receptor. Research has shown a similar response to direct-binding asbestos fibers. The proliferative effects are signaled through several pathways. Crosstalk of EGF signaling with other pathways make the EGF receptor a junction point between signaling systems.

Variability Among Fiber Types

- **Serpentine (Chrysotile) versus Amphibole Asbestos fibers:**
  - Chrysotile has been shown to produce less ROS and a decreased magnitude of cellular damage.
  - Chrysotile yields increased levels of RNS (nitric oxide, peroxynitrite) compared to crocidolite.
  - Effect of other varied characteristics relating to solubility, fiber splitting, surface charge.
- **Long (>10um) vs short fibers (<5um):**
  - The longer fibers have been shown to lead to increased aneuploidy and chromosomal aberrations. These fibers are able to interact with the mitotic spindle and alter cell cycle progression.
  - Once engulfed by the macrophage, small fibers may remain in place or may be removed across the bronchiole or alveolar membrane and transported to the interstitial and pleural space through the blood vessels or lymphatic system.

Toxicity Pathway Analysis

- **Exposure Pathways**
- Asbestos studies have demonstrated increased activation of many signaling pathways following exposure to various types of asbestos.
- Signaling activation results in apoptosis, cell proliferation, cell cycle alterations and enzyme activation.
- ROS production activates a variety of signaling cascades (MAPK, NFkB, ERK1/2, PI3K).
- Differences in the magnitude and type of signaling response may be related to fiber type and size.
- **Disease Pathways**
- Understanding of the varied signaling pathways involved in adverse health endpoints will aid in determining differences in response to fibers.
- Biomarkers of exposure and/or disease can be used to inform mode of action analysis.
- Linking toxicity pathways for different disease endpoints to determinants of fiber toxicity will help define variable responses to all fiber types.

Data Gaps and Next Steps

- Further understanding the variability between different fibers types is necessary to clarifying what characteristics of asbestos result in adverse health effects, including cancer. Questions still to be answered include:
  - *Are differences in response related to activation of different pathways, or variable magnitude of response of the same pathway?*
  - *Is there an additive or synergistic effect of exposure to one type of asbestos fibers related to different aspects of fiber composition?*
- Toxicogenomics, and other –omics technologies, can be used to link determinants of fiber toxicity to disease endpoints.
- Comparative studies of various fiber types and target tissues should be done to address the questions above.
- Gene profile signatures for asbestos-induced disease endpoints can inform asbestos mode of action.
- Recent studies have looked at gene expression profiles for mesothelioma (Roe et al. 2009) and issues with in vitro studies of various pathogenic particle types (Donaldson et al. 2009). More studies like this will help to define the toxicity pathways involved in exposure and disease related to asbestos.
- Increased research is needed to fully understand the causal role of individual fiber characteristics to elucidate the mechanism of action of all asbestos fibers and how they lead to differential response.

- *References available upon request.*