MECHANISMS BY WHICH FIBERS CAUSE PATHOLOGY

Val Vallyathan and Vince Castranova
National Institute for Occupational Safety and Health
Pathology and Physiology Branch,
Health Effects Laboratory
Morgantown, WV

Preamble

This review will briefly address the mechanisms by which asbestos fibers cause pathology. Characteristic physical and chemical features of asbestos are considered important in pathogenesis and are briefly discussed in this overview. Detailed information on many of these important physical, chemical and bio-durability properties are available in many reviews cited. This overview on the mechanisms by which asbestos fibers cause pathology is presented as background information which may be applicable for the early identification of potential emerging hazards resulting from the introduction of new technologies in the fiber industry.

Introduction

Epidemiologic, pathologic, and experimental studies have established that inhalation of asbestos induces asbestosis, bronchogenic carcinomas, malignant mesothelioma of the pleura and peritoneum, and several other adverse effects (1-5). In addition to the direct target organ interaction and development of pulmonary fibrosis, the ability of asbestos to induce other malignant diseases in different cell types is enigmatic. The biochemical mechanisms involved in these processes are thought to be divergent and are related to physical characteristics, chemical composition, surface reactivity and bio-durability of the specific type of asbestos fibers (1-5). An important fiber characteristic is the bio-durability, or persistence of fibers in the tissues, and their physical movement within the body. In addition, additive or synergistic relationships between asbestos exposure and cigarette smoking are known to be major factors in carcinogenesis.

Asbestos Mineralogy

Asbestos has been known since antiquity for its remarkable fireproofing qualities, strength and durability. It was only since 1877 that commercial exploitation began. Its use escalated to six to eight million tons during World War II in U.S. Asbestos is a group of fibrous silicate minerals which can be classified into two basic subgroups known as serpentines and amphiboles (1). Chrysotile, a serpentine asbestos, is white, curly and flexible. It accounts for 95% of the asbestos used in the United States and Canada and is mined mainly in Canada and the USSR. Amphiboles are chain silicates, in contrast to serpentines, which have a layered structure. Amphiboles, such as crocidolite (blue asbestos), amosite (brown asbestos), tremolite and actinolite, are rod-shaped, thin, cylindrical fibers. Commercial use of amphiboles has declined in recent years. Amphiboles are more durable and persistent in the lung than the chrysotile asbestos which gradually leaches in the body fluids and become less bio-durable. The bio-durability of the amphiboles with the straight rod like physical properties is correlated with the greater pathogenicity of these fibers. Asbestos generally separates longitudinally into finer and finer fibrils during processing. The aerodynamic properties together with the indestructible, rod-like shape of amphiboles are apparently related to their greater pathogenicity and ability to reach intra- and extra-pulmonary sites and other organs.

Asbestosis

Asbestos-related diseases, such as asbestosis, lung cancer and mesothelioma were unknown 65-70 years ago. Now they are responsible for major medical and legal problems. It was estimated that at least 27-28 million U.S. workers have been exposed to asbestos, resulting in at least 8000 asbestos-related cancer deaths per year (6).

Asbestos exposure is known to cause a bilateral diffuse interstitial pulmonary fibrotic disease known as asbestosis. Asbestosis is directly related to the intensity and duration of asbestos exposure. Asbestosis initially begins as fibrosis around respiratory bronchioles and alveolar ducts associated with the presence of asbestos bodies (1, 5). As the fibrosis advances, adjacent alveolar ducts and terminal bronchioles are affected in a centrifugal fashion involving the lung parenchyma extensively. In advanced disease, thick fibrous walls are formed with the development of a honeycombed lung (1). Clinically, asbestosis is characterized by a restrictive pattern with reduction in diffusing capacity. Radiographically, asbestosis appears as irregular or linear opacities usually in the lower lung lobes with or without pleural thickening. Pleural plaques, hallmarks of past asbestos exposure, are frequently present along the lower lung fields and they are usually benign.

Experimental studies indicate that during phagocytosis of the asbestos several biochemical pathways are triggered, resulting in the generation of reactive oxygen species (ROS), stimulation of inflammatory events, lipid peroxidation, generation of reactive lipid intermediates, secretion of cytokines, chemokines, fibrogenic growth factors and membrane injury which causes the release of hydrolytic enzymes from lysosomes (3, 5, 7-11). Various types of asbestos contain different percentages of iron {>amosite (34%), >crocidolite (18%), > chrysotile (1%)}. In the presence of hydrogen peroxide, the iron on the surface of asbestos can catalyze the generation of hydroxyl radicals. Bio-durability and the chronic generation of ROS

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 1:148-151 (1999)
National Cotton Council, Memphis TN

become important factors in the pathogenesis of disease (2,3,5,12).

Bio-durability and the ability to generate ROS on a persistent basis leads to chronic inflammation and increased secretions of cytokines, which cause cell proliferation and oxidative stress. Epithelial cell proliferation can lead to lung cancer while fibroblast proliferation results in fibrogenesis. Following two short-term inhalation exposures to chrysotile asbestos in experimental animals, proliferative changes were observed in bronchial epithelial cells and alveolar cells. It is known that the initiation of proliferation in the epithelial cells and fibroblasts occurs after the activation and induction of early response genes such as c-*fos*, c-*ju*n and c-*myc* (3, 13-15). These genes can induce activator protein-1 (AP-1) which is important in inflammation, proliferation, cell division and apoptosis. Increased expression of AP-1 was also observed in AP-1 reporter mice intratracheally instilled with asbestos (16).

Bronchogenic Carcinoma

The risk for lung cancer in asbestos workers is associated with a latency period of 20 or more years after the first exposure. Lung cancer arises more frequently in the major conducting airways and is typically of epithelial origin. In asbestos workers without any cigarette smoking history, there is an increased risk for lung cancer associated with the severity and type of asbestos exposure (1, 3, 5, 17,18). However, there is conclusive evidence indicating that cigarette smoking acts to increase the risk of lung cancer in asbestos workers (17-21). It appears that multiplicative a or synergistic effect is involved in the increased risk of lung cancer in smoking asbestos workers. Adsorption of carcinogens from cigarette smoke onto asbestos fibers may be an important factor in the synergy and increased incidence of lung cancer in smoking asbestos workers (21). Therefore, in the development of a bronchogenic carcinoma, asbestos appears to function as a tumor promoter or co-carcinogen. In this respect, the types of asbestos involved is of specific interest. Bronchogenic carcinomas occur following exposure to all the major types of asbestos (crocidolite, amosite, tremolite, chrysotile). The incidence of cancer appears to be associated with the physical characteristics, chemical composition and surface properties of asbestos. It is proposed that iron-rich bio-durable asbestos fibers, such as crocidolite and amosite, may produce excess amounts of ROS during frustrated phagocytosis promoting chronic inflammation which is causally linked to carcinogenesis.

Asbestos is classically a non-genotoxic agent. It is negative in Ames assays and in rodent bone marrow assays for chromosomal abnormalities and micronucleus formation. However, asbestos fibers are capable of inducing mutagenicity, chromosomal changes and deletion mutations in a human-hamster hybrid assay (22-25). These effects were inhibited by antioxidants, thereby suggesting that they were mediated through asbestos-catalyzed ROS mechanisms. Amphibole asbestos was also shown to induce increased ROS-induced oxidative damage and strand breaks in DNA. In bronchial epithelium, asbestos can induce a dose-dependent increase in ornithine decarboxylase, a rate limiting enzyme involved in the biosynthesis of polyamines, which plays a pivotal role in cell proliferation (26). Similarly, protein kinase C, a receptor of tumor promoters, is also induced by asbestos in bronchial epithelial cells in a dose-dependent manner (27). It is known that proto-oncogenes, such as c-*myc* and c-*fos*, are potential targets of protein kinase C action and may induce genes associated with growth factors thereby promoting cell proliferation. These characteristic responses to asbestos in the bronchial epithelium are typical of chemical tumor promoters. It can be inferred from these studies that asbestos in the bronchial epithelium acts as a tumor promoter rather than a direct carcinogen.

Available experimental evidence suggests that asbestos can act indirectly in association with other co-carcinogens. Asbestos fibers probably acts as a carrier for polycyclic aromatic hydrocarbons, such as benzo-(a) pyrene derived from cigarette smoke (19, 21). Asbestos fibers with a large surface area would provide effective transfer and sustained interaction of carcinogens adsorbed from tobacco smoke. Although oncogenes are known to be intrinsically involved with the development of tumors, no asbestos-associated molecular patterns have been identified in human lung cancers in contrast to the presence of such alterations in human mesotheliomas.

Mesotheliom

Mesothelioma is a rare tumor arising from the single layer of mesothelial cells lining the pleura and peritoneum. It is considered to be a signal neoplasm associated with exposure to asbestos. Mesothelioma develops after a long latency period generally 30-40 years following the first exposure (1, 2, 5). In contrast to bronchogenic carcinomas, an increased risk of mesothelioma does not appear to be associated with cigarette smoking. Short heavy exposures of the environmental or avocational type have been associated with the development of mesothelioma. Mesothelioma is induced by the asbestos fibers reaching the pleural and peritoneal cavities. Mesothelioma of the peritoneum is usually associated with severe exposure history while that of the pleura is generally associated with chronic moderate exposure. As in the case of lung cancer, mesothelioma also appears to be more frequently associated with specific types of asbestos fiber. This again appears to be associated with physical chemical characteristics, bio-durability and potential to produce ROS on a persistent nature. Mesothelioma induction and its relation to fiber size were extensively investigated by Stanton et al (28). They concluded that thin long fibers were much more

potent in the induction of mesothelioma in animals than short fibers.

In the induction of mesothelioma, asbestos appears to function as a complete carcinogen for mesothelial cells. Mesothelial cells appears to be very sensitive to the direct effects of asbestos. Further support for this sensitivity of mesothelial cells is evident from studies with weakly carcinogenic chrysotile or non-carcinogenic fiber glass in man that are potent mesothelial carcinogens in experimental animals (27, 29). A wide variety of cytogenitic alterations are reported with asbestos fibers. These include polysomy, monosomy, aneuploidy and other clastogenic effects. Chromosomal alterations and mitotic interferences in the mesothelial cells are probably caused by the penetration of asbestos fibers and fiber-catalyzed ROS reactions. Human mesothelioma shows altered patterns of oncogenes and tumor suppressor genes which are different than that of bronchogenic carcinomas (30)..

Other Fibrous Minerals

As with asbestos, pathological responses to other fibers, such as erionite fiber glass depend on physical chemical characteristics, bio-durability and ability to produce ROS on a chronic basis. It is known that similar to asbestos erionite fibers cause pleural plaques, pleural effusions, diffuse fibrosis and malignant mesothelioma in humans. Ceramic fibers and fiber glass also produce maliganant reactions in experimental animals.

From the results of in vitro and in vivo studies with fibers a schematic diagram showing some of the major mechanisms involved in fibrogenesis, carcinogenesis and induction of mesothelioma can be constructed (Figures 1 and 2).

References

1. Craighead JE, Abraham JL, Churg A, Green FHY, Kleinerman J, Pratt P, Seemayer TA, Vallyathan V, and Weill H. Asbestos-associated diseases. Arch Path Lab Med 106:540-597, 1982.
2. Mossman BT and Churg A. Mechanisms in the pathogenesis of asbestosis and silicosis. Am J Respir Crit Care Med 157:1666-1680, 1998.
3. Mossman BT, Kamp DW, and Weitzman SA. Mechanisms of carcinogenesis and clinical features of asbestos-associated cancers. Can Res 14:466-480, 1996.
4. IARC Scientific Publications No. 140, Mechanisms of Fibre Carcinogenesis, 1996, (Eds. Kane AB, Bofetta P, Saracci R, and Wilbourn JD), Lyon, France, pp135.
5. Churg A. Nonneoplastic Disease Caused by Asbestos. In: Pathology of Occupational Lung Disease. (Eds Churg A and Green FHY, Second Edition) 1998, Williams and Wilkins, Philadelphia, PA, pp 277-338.
6. National Occupational Research Agenda, National Institute for Occupational Safety and Health, U.S. Department of Human Health and Human Services, DHHS(NIOSH) Publication No. 96-115, 1996, pp 38-39.
7. Vallyathan V, Mega JF, Shi X, and Dalal NS. Enhanced generation of free radicals from phagocytes induced by mineral dusts. A J Respir Cell Mol Biol 6: 404-413, 1992.
8. Quinlan TR, Marsh JP, Jansseen YMW, Borm PA, and Mossman BT. Oxygen radicals and asbestos-mediated disease. Enivron Health Perspect 102 (10): 107-110, 1994.
9. Moyer VD, Cistulli CA, Vaslet CA, and Kane AB. Oxygen radicals and asbestos carcinogenesis. Enivron Health Perspect 102 (10): 131-136, 1994.
10. Jackson JH, Schraufstatter IU, Hyslop PA, Vosbeck K, Sauerheber R, Weitzman SA, Cochrane CG. Role of oxidants in DNA damage: hydroxyl radical mediates the synergistic DNA damaging effects of asbestos and cigarette smoke. J Clin Invest 80: 1090-1095, 1987.
11. Mossman BT, Bignon J, Corn M et al Asbestos: scientific developments and implications for public policy. Science 247: 294-301, 1990.
12. Bellman B, Koning H, Muhle H, and Pott F. Chemical durability of asbestos and of man-made mineral fibres in vivo. J Aerosol Sc 17: 341-345, 1986.
13. Janssen YM, Matalon WS, and Mossman BT. Differential induction of c-fos, c-jun, and apoptosis in lung epithelial cells exposed to to ROS or RNS. Am J Physiol 17: L789-L796, 1997.
14. Heintz NH, Janssen YM, and Mossman BT. Persistent induction of c-fos and c-jun protooncogenes expression by asbestos. Proc Natl Acad Sci U.S.A. 90:3299-3303, 1993.
15. Janssen YM, Heintz NH, Marsh JP, Born PJA, and Mossman BT. Induction of c-fos and c-jun protooncogenes in target cells of the lung and pleura by carcinogenic fibers. Am J Respir Cell Mol Biol 11:522-530, 1994.
16. Ding M, Dong Z, Chen F, Pack D, Ma WY, Ye J, Shi X, Castranova V, and Vallyathan V. Asbestos induces activator protein-1 (AP-1) transactivation in transgenic mice. Cancer Research (In press).
17. Ernster VL, Mustacchi P, and Osann KE.Epidemiology of lung cancer. In: Textbook of Respiratory Medicine, Vol II (Eds. Murray JF and Nadel JA), 1994, WB Saunders, New York, NY, pp1504-1527.
18. Hammond EC, Selikoff IJ and Seidman H. Asbestos exposure, cigarette smoking and death rates. Ann NY Acad Sci 330: 473-492, 1979.
19. Reiss B, Tony C, Telang S, and Williams GM.. Enhancement of benzo[a] pyrene mutagenicity by chrysotile asbestos in rat liver epithelial cells. Environ Res 31: 100-104, 1983.

20. McFadden D, Wright JL, Wiggs B, and Churg A. Smoking inhibits asbestos clearance. Am Rev Respir Dis 133:372-374, 1986.
21. Churg A. Neoplastic Asbestos-Induced Disease. In: Pathology of Occupational Lung Disease. (Eds Churg A and Green FHY, Second Edition) 1998, Williams and Wilkins, Philadelphia, PA, pp 339-391.
22. Hei TK, He ZY, Piao CQ, and Waldren C. The mutagenicity of mineral fibers. In: Mechanisms of Fiber Carcinogenesis, 1991, (Ed. Brown RC) Plenum Publishing Corporation, New York, NY, pp 319-325.
23. Yegles M, Saint-Etienne L, Renier A et al Induction of metaphase and anaphase/telophase abnormalities by asbestos fibers in rat pleural mesothelial cells in vitro. Am J Respir Cell Mol Biol 9: 186-191, 1993.
24. Pelin K, Husgafvel-Persiainen K, Vallas M et al Cytotoxicity and anaphase aberrations induced by mineral fibers in cultured human mesothelial cells. Toxic In Vitro 6: 445-450, 1992.
25. Jaurand MC. Mechanisms of fibre genotoxicity, 1991, In: Mechanisms in fibre carcinogenesis (Ed. RC Brown, JA Hoskins and NF Johnson) Plenum Press, New York, NY pp287-307.
26. Marsh JP, Mossman BT. Mechanisms of induction of ornithine decarboxylase activity in tracheal epithelial cells by asbestiform minerals. Cancer Res 48: 709-714, 1988.
27. Perderiset M, Marsch JP, and Mossman BT. Activation of protein kinase C by crocidolite asbestos in hamster epithelial cells. Carcinogenesis 12: 1499-1502, 1991.
28. Stanton MF, Layard M, Tegeris A et al Relation of particle dimention to carcinogenicity in amphibole asbestos and other fibrous minerals. J Natl Cancer Inst 67: 965-975, 1981.
29. Wagner JC, Berry G, Timbrell V. Mesotheliomas in rats after innoculation with asbestos and other minerals. Br J Cancer 28: 173-185, 1973.
30. Lechner JF, Tesfaigzi J, and Gerwin BI. Oncogenes and tumor supressor genes in mesothelioma: a synopsis. Environ Health Perspect Suppl 105: 1061-1068, 1997.