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Asbestos: Don't Inhale

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Abbreviations:

AP-1, activator protein-1
DCF-DA, 2'-7' dichlorodihydrofluorescein
DHE, dihydroethidium
DMPO, 5,5'-dimethyl-1-pyrroline-N-oxide
DNA-SB, DNA-Strand Break
iNOS, inducible nitric oxide synthase
MAPK, mitogen-activated protein kinases
NADPH, nicotinamide adenine dinucleotide phosphate
NF-kB, nuclear factor kappa B
NMMA, NG-monomethyl-L-arginine
TBARS, thiobarbituric acid reactive substances
8-OHdG, 8-hydroxy-2'-deoxyguanosine (8-OHdG)
$ONOO^-$, peroxynitrite
4-POBN, α–(4-pyridyl-1-oxide)-*N*-*tert*-butylnitrone
RNS, reactive nitrogen species
ROS, reactive oxygen species

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Abstract

Asbestos is a generic term for a group of six naturally occurring silicate fibers, including amosite, crocidolite, tremolite, anthophyllite, actinolite (amphibole class) and chrysotile (serpentine class). These two classes of asbestos fibers have been linked to various pulmonary and pleural disorders and malignancies, such as asbestosis and malignant mesothelioma. The mechanism implicated in asbestos-induced toxicity and carcinogenicity involves the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS). Asbestos cause cellular damage by targeting critical biomolecules, such as lipid membranes, DNA and signal transduction proteins. In this mini-review, the genotoxic properties of asbestos, its mechanism of action, and methods of detection are discussed.

Introduction

Asbestos is a group of naturally occurring hydrated silicate fibers that have ≥3:1 length to diameter ratio. The basic unit of asbestos is the silicate (SiO_4) group [1]. The silicate group can form various polymeric structures through the formation of Si-O-Si bonds. Depending on their structure, asbestos is divided into two classes: amphibole and serpentine. The amphibole class of asbestos includes amosite, crocidolite, tremolite, anthophyllite, and actinolite (**Table 1**) [2].

CLASS	TYPE	CHEMICAL STRUCTURE
Serpentine	Chrysotile	$[Mg_6Si_4O_{10}(OH)_8]$
Amphibole	Crocidolite	$[Na_2(Fe^{3+})2(Fe^{2+})_3Si_8O_{22}(OH)_2]$
	Amosite	$[(Fe,Mg)_7Si_8O_{22}(OH)_2]$
	Anthophyllite	$[(Mg,Fe)_7Si_8O_{22}(OH)_2]$
	Actinolite	$[Ca_2(Mg,Fe)_5\text{-}Si_8O_{22}(OH)_2]$
	Tremolite	$[Ca_2(Mg_5Si_8O_{22}(OH)_2]$

Table 1. Types of asbestos fibers and their chemical structures [2].

Amphibole asbestos is characterized by linear double chains that crystallize into long, thin, straight fibers (**Figure 1**). The serpentine family only includes chrysotile whose polymeric form is an extended sheet that wraps around itself to form a tubular fiber structure. Chrysotile fibers tend to be curved into serpentine-like shape in contrast to the straight morphometry of amphibole fibers [1,2]. Asbestos is associated with the development of malignant and nonmalignant illnesses in lungs and pleura [3]. The mechanism implicated in asbestos-induced toxicity and carcinogenicity includes generation of iron-derived reactive oxygen species (ROS) and reactive nitrogen species (RNS). In this mini-review, asbestos-induced production of free radical species

and the methods of their detection are discussed. Then, the role of ROS and RNS in the induction of asbestos-associated diseases is reviewed.

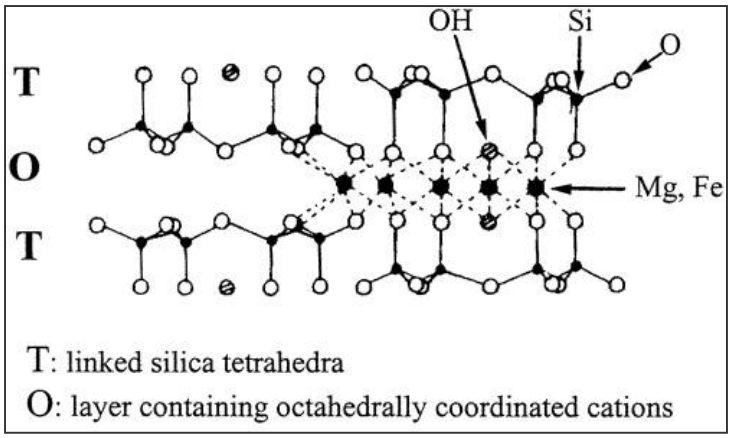

Figure 1. Typical amphibole structure showing a double chain of linked silica tetrahedra that form the asbestos axis [1].

Mechanism of Action

Asbestos mechanism of action involves generation of ROS and RNS species.

A. Asbestos Increase Generates ROS

Asbestos fibers are able to generate hydroxyl radical ($HO^{\bullet}$) by reducing oxygen and by participating in a Fenton-type reaction. Asbestos fibers reduce O_2 to $O_2^{\bullet-}$ radical, which is then dismutated to produce H_2O_2. Hydrogen peroxide is then decomposed to form $HO^{\bullet}$ in a Fenton-type reaction shown in the following reaction [4]:

$$O_2 \xrightarrow{+ e^-} O_2^{\bullet-} \qquad \text{(Reaction 1)}$$

$$O_2^{\bullet-} \xrightarrow{+ e^- + 2H^+} H_2O_2 \qquad \text{(Reaction 2)}$$

$$H_2O_2 \xrightarrow{+ e^-} HO^{\bullet} + HO^- \qquad \text{(Reaction 3)}$$

Hydroxyl radical either generates water or reacts with various molecules to produce carbon-centered radicals, $R^{\bullet}$, or lipid radicals, $L^{\bullet}$, as illustrated in Reaction 4-6 [4]:

$$HO^{\bullet} \xrightarrow{+ e^{-} + H^{+}} H_2O \qquad \text{(Reaction 4)}$$

$$HO^{\bullet} + RH \longrightarrow H_2O + R^{\bullet} \qquad \text{(Reaction 5)}$$

$$HO^{\bullet} + LH \longrightarrow H_2O + L^{\bullet} \qquad \text{(Reaction 6)}$$

Asbestos fibers contain iron, which is thought to play an important role in the production of $HO^{\bullet}$ through either the reduction of O_2 or the decomposition of H_2O_2. Total iron content does not affect the ability of asbestos fibers to generate free radicals. However, the specific iron active sites located at the surface or the core structure of asbestos become active during free radical production and catalyze the formation of $HO^{\bullet}$ by the Haber-Weiss reaction as shown in the following reaction [3]:

$$Fe(II) + H_2O_2 \longrightarrow OH^{-} + HO^{\bullet} + Fe(III) \qquad \text{(Reaction 6)}$$

$$O_2^{\bullet -} + H_2O_2 \longrightarrow \text{Iron} \longrightarrow HO^{\bullet} + OH^{-} + O_2 \qquad \text{(Reaction 7)}$$

$$O_2 + Fe(II) \longrightarrow O_2^{\bullet -} + Fe(III) \qquad \text{(Reaction 8)}$$

Serpentine fibers have low iron content (≈6%) but amphibole fibers have high iron content (27%-33%). Redox-active iron in asbestos has the ability to induce synthesis of apoferritin for iron storage [3]. In addition to ROS generated by asbestos fibers, phagocytic cells undergoing frustrated uptake of asbestos are another abundant source of ROS. Asbestos can induce these cells to generate $HO^{\bullet}$, H_2O_2 or $O_2^{\bullet -}$ either through nicotinamide adenine dinucleotide phosphate (NADPH) oxidase or mitochondrial dysfunction [2].

B. Asbestos Generates RNS

Nitric oxide ($NO^{\bullet}$) is a universal signaling molecule, which is generated from arginine by constitutive or inducible nitric oxide synthase (iNOS) present in cells [4]:

$$\text{L-Arginine} \longrightarrow O_2 \,/\, \text{NADPH} \,/\, \text{NOS} \longrightarrow \text{L-Citriline} + NO^{\bullet} \qquad \text{(Reaction 9)}$$

Asbestos has been shown to induce the expression and activity of iNOS in alveolar macrophages and mesothelial cells. Once $NO^{\bullet}$ is formed, it can interact with $O_2^{\bullet -}$ to form peroxynitrite ($ONOO^-$), a highly reactive oxidant that attacks a variety of biological targets [4]:

$$NO^{\bullet} + O_2^{\bullet -} \rightarrow ONOO^- + H^+ \rightarrow ONOOH \rightarrow NO_2^{\bullet} + HO^{\bullet} \qquad \text{(Reaction 10)}$$

Indeed, alveolar macrophages isolated from rats that have inhaled crocidolite or chrysotile asbestos fibers were found to release nitrite (NO_2^-) and nitrate (NO_3^-). Addition of iNOS inhibitor NG-monomethyl-L-arginine (NMMA) inhibited the release of RNS [5]. In addition, crocidolite or chrysotile asbestos fibers were found to increase iNOS protein expression in rat pleural mesothelial cells *in vitro*. The fact that asbestos activates iNOS expression in various cells of the lung indicates that free radicals derived from $NO^{\bullet}$ regulate pulmonary toxicity. However, $NO^{\bullet}$ also attenuates H_2O_2-induced lipid peroxidation and pulmonary artery endothelial cell injury, suggesting that is has antioxidant functions [4]. Thus, there is a complex balance between protective and damaging effects of RNS species induced by asbestos that needs to be further investigated.

Molecular Targets of ROS and RNS Induced by Asbestos

Asbestos-derived ROS and RNS species contribute to genotoxic effects of asbestos, which include DNA damage and apoptosis. Several lines of convincing evidence shows that ROS/RNS generated either by asbestos fibers directly or activated phagocytic cells induce cellular damage. Schapira and colleagues demonstrated that asbestos generates $HO^{\bullet}$ in rat lungs one week after exposure to chrysotile asbestos [6]. Antioxidants, such as catalase and superoxide dismutase (SOD), attenuated asbestos-induced toxicity in pulmonary epithelial cells, mesothelial cells and pulmonary epithelial cells [4]. Asbestos fibers caused apoptosis in alveolar macrophages and mesothelial cells. Apoptosis, also known as programmed cell death, is an important mechanism

by which damaged cells can be eliminated without triggering an inflammatory response. Asbestos and its second messengers, ROS and RNS, cause cellular damage by targeting critical biomolecules, such as lipid membranes, DNA and signal transduction proteins [4].

A. Lipid Membranes

Asbestos modifies cell membrane and function by causing lipid peroxidation. Asbestos-associated iron catalyzes the formation of lipid peroxidation products. Antioxidants and iron chelators reduce lipid peroxidation, which underscores the important role of iron-catalyzed ROS/RNS in asbestos-mediated lipid peroxidation [7]. Furthermore, lipid peroxidation products are present in the plasma of workers exposed to asbestos as measured by thiobarbituric acid reactive substances (TBARS) [8].

B. DNA

Asbestos can induce cell injury by damaging DNA. The ability of asbestos to damage the genetic material has been demonstrated in cell free systems, as well as in asbestos target cells like pulmonary epithelial cells and pleural mesothelial cells [4]. DNA damage caused by asbestos manifests as DNA-Strand Break (DNA-SB) formation, altered DNA bases, apoptosis and chromosomal aberrations. Hydroxyl radical and $ONOO^-$ commonly react with DNA to generate hydroxylated bases and DNA-SB and so have been credited specifically with altering DNA base pairs. Asbestos promotes the formation of 8-hydroxy-2'-deoxyguanosine (8-OHdG), a modified DNA base that has been implicated as a potentially carcinogenic DNA lesion [9].

C. Signal Transduction Proteins

Asbestos and ROS/RNS modify cellular function by activating signal transduction proteins, including mitogen-activated protein kinases (MAPK) and transcription factors, such as nuclear factor kappa B (NF-kB) and activator protein-1 (AP-1). Transcription factors like NF-kB tightly

regulate a number of genes, including cytokines and NOS. Involvement of asbestos-induced ROS/RNS species in regulation of NF-kB is evidenced by the observation that oxidants (H_2O_2) stimulate NF-kB activity while antioxidants decrease NF-kB activation [10].

Detection of Asbestos-Derived ROS and RNS

Two common methods used to detect asbestos-derived ROS and RNS species are electron spin resonance (ESR) and fluorescence.

Electron Spin Resonance (ESR)

In electron spin resonance (ESR) spin trapping method, the stable spin adducts 5,5'-dimethyl-1-pyrroline-N-oxide (DMPO) or α–(4-pyridyl-1-oxide)-*N-tert*-butylnitrone (4-POBN) were used to detect free radical generation by asbestos in rats [11,12]. Rats were intratracheally administered either saline or 500 μg crocidolite asbestos. Twenty-four hours later, chloroform extract from the lungs of rats exposed to asbestos were analyzed by ESR spectroscopy (**Figure 2**).

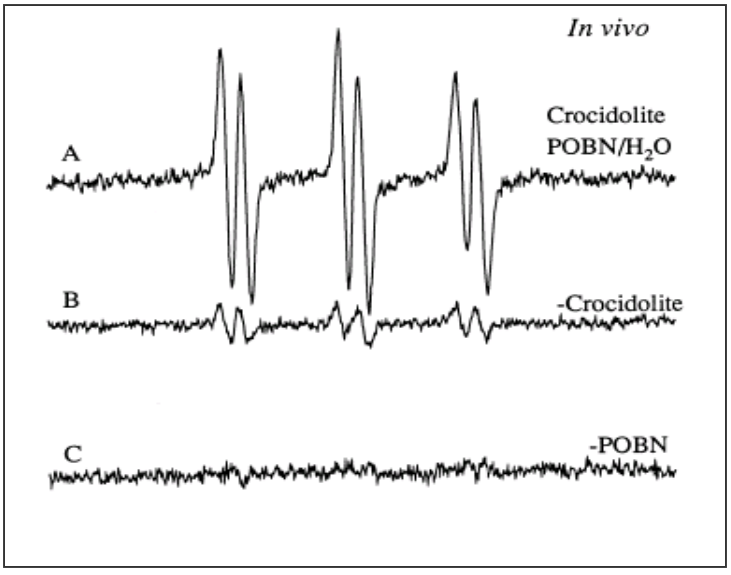

Figure 2. (A) ESR spectrum of 4-POBN radical adducts detected in lipid extracts of rat lungs 24h after asbestos instillation and 1h after intraperitoneal administration of 4-POBN. B) same as (A) but rats were not instilled with asbestos. C) same as (A) but rats were not administered 4-POBN [11,12].

Those spectra revealed a carbon-centered radical adduct in rats exposed to asbestos but not rats administered saline. This same radical formation persisted one month after asbestos instillation. Collectively, these data provide evidence for *in vivo* free radical formation caused by asbestos exposure [11,12].

Chemiluminescence Assay

ROS and RNS generated due to asbestos exposure have been studied using two fluorescent markers of cellular oxidant production, 2'-7' dichlorodihydrofluorescein diacetate (DCF-DA) and dihydroethidium (DHE) [13]. DCF-DA and DHE are freely diffusible and are oxidized to their fluorescent derivatives by various oxidant species. Cells are usually loaded with either DCF-DA or DHE and then exposed to asbestos fibers. Cells generating ROS become fluorescent. These chemiluminescence assays are nonspecific but provide a general indication of oxidation [13]. In fact, direct measurements of ROS/RNS in asbestos-exposed cells are very difficult, which is the reason why ROS-mediated reactions in asbestos-treated cells are still poorly understood.

Summary

In this mini-review, the properties and the molecular mechanisms underlying asbestos-induced pulmonary disorders are discussed. Considerable evidence suggests that ROS and RNS species play a key role in causing asbestos-associated pulmonary toxicity. The evidence also shows that iron associated with the core or the surface structure of asbestos catalyzes the production of $HO^{\bullet}$. Additionally, the evidence reviewed here suggests that asbestos and ROS and RNS, acting as its second messengers, cause cellular damage by targeting pivotal biological macromolecules, such as lipids, DNA and signaling proteins. The methods used to detect ROS/RNS-derived from asbestos are described, including ESR spin trapping and chemiluminescence.

References

1. Hanley DG, Kess S, Stevens YW, Wilbur S, Williams M. (1999) Chemical and physical properties of asbestos. In: *ATSDR Toxicological Profiles*. Vol. 5. Boca Raton, FL: CRC Press LLC; pp135-155.

2. Upadhyay D, Kamp DW. (2003) Asbestos-induced pulmonary toxicity: role of DNA damage and apoptosis. *Exp Biol Med*. **228**:650-659.

3. Shukla A, Gulumian M, Hei TM, Kamp DW, Rahman Q, Mossman BT. (2003) Multiple roles of oxidants in the pathogenesis of asbestos-induced diseases. *Free Radic Biol Med*. **34**:1117-1129.

4. Kamp DW, Weitzman SA. (1999) The molecular basis of asbestos induced lung injury. *Thorax*. **54**:638-652.

5. Quinlan TR, Berube KA, Hacker MP. (1998) Mechanisms of asbestos induced nitric oxide production by rat alveolar macrophages in inhalation and in vitro models. *Free Radic Biol Med*. **24**:778-788.

6. Schapira RM, Ghio AJ, Effros RM. (1994) nHydroxyl radicals are formed in the rat lung after asbestos instillation *in vivo*. *Am J Respir Cell Mol Biol*. **10**:573-579.

7. Kamp DW, Graceffa P, Prior WA. (1992) The role of free radicals in asbestos induced diseases. *Free Radic Biol Med*. **12**:293-315.

8. Kamal A-AM, Gomas A, Khafif ME. (1989) Plasma lipid peroxides among workers exposed to silica or asbestos dusts. *Environ Res*. **49**:173-180.

9. Chao CC, Park SH, Aust AE. (1996) Participation of nitric oxide and iron in the oxidation of DNA in asbestos-treated human lung epithelial cells. *Arch Biochem Biophys*. **326**:152-157.

10. Blackwell TS, Christman JW. (1996) The role of nuclear factor-kB in cytokine gene regulation. *Am J Respir Cell Mol Biol*. **17**:3-9.

11. Gulumian M, Van Wyk JA. (1987) Hydroxyl radical production in the presence of fibres by a Fenton-type reaction. *Chem Biol Interact*. **62**:89-87.

12. Ghio AJ, Kadiiska MB, Xiang QH, Mason RP. (1998) In vivo evidence of free radical formation after asbestos instillation: an ESR spin trapping investigation. *Free Radic Biol Med*. **24**:11-17.

13. Kopnin PB, Kravchenko IV, Furalyov VA, Pylev LN, Kopnin BP. (2004) Cell type-specific effects of asbestos on intracellular ROS levels, DNA oxidation and G1 cell cycle checkpoint. *Oncogene*. **23**:8834-8840.