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Particulates and the Airways: Basic Biological Mechanisms of Pulmonary Pathogenicity

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With strong evidence that materials such as silica and asbestos are associated with irreversible lung disease, efforts have been made to identify abrasive substitute materials for silica and produce various synthetic vitreous fibers as substitutes for asbestos. With the introduction of new materials, questions concerning potential health effects are raised. A clear understanding of the mechanisms by which certain inhaled particles induce lung damage and subsequent disease is vital to the ability to predict the relative safety of new materials, as well as, to provide appropriate measures for the safe use of old materials currently grouped together as particulates not otherwise classified/regulated. The following is a brief review of mechanisms currently thought to be important in the development and progression of particle-induced lung disease. CASTRANOVA, V.: PARTICULATES AND THE AIRWAYS: BASIC BIOLOGICAL MECHANISMS OF PULMONARY PATHOGENICITY. APPL. OCCUP. ENVIRON. HYG. 13(8):613–616; 1998. © 1998 AIH.

As technology develops, new materials are introduced into the workplace. Often these materials are developed to serve as substitutes for substances known to be associated with increased risk of occupational lung disease. In most cases the relative cytotoxicity and pathogenicity of these substitute materials are not clearly defined. Therefore, safety and health professionals are forced to extrapolate from incomplete data to predict the relative safety of these substances. To make these predictions sound, an understanding of the mechanisms by which particles initiate adverse pulmonary responses is essential. An understanding of such mechanisms would also assist in determining whether certain materials currently classified as particulates not otherwise classified/regulated (PNOC/R) require new exposure limits to prevent occupational lung disease.

Currently four basic mechanisms have been proposed to explain pulmonary pathogenesis following inhalation of certain particles:

1. Direct cytotoxicity: The particle is directly toxic to lung cells, causing lung damage and scarring.
2. Activation of phagocytes: The particle stimulates the release of reactive oxidant species from pulmonary phagocytes, such as alveolar macrophages. These oxidants cause lung damage and resultant scarring.
3. Secretion of inflammatory cytokines: The particle stimulates the release of chemokines from alveolar macrophages and/or alveolar epithelial cells which recruit polymorphonuclear leukocytes from the pulmonary capillaries to the air spaces. Once in the air spaces, these leukocytes would release reactive species, increasing the oxidant burden in the lung and causing damage and scarring.
4. Secretion of proliferative factors: The particle stimulates the release of growth factors from alveolar macrophages and/or alveolar epithelial cells which stimulate fibroblast proliferation and induce fibrosis.

Direct Cytotoxicity

Evidence indicates that certain pathogenic particles are directly toxic to lung cells. Such particles can cause lipid peroxidation of cell membranes, which can result in a loss of membrane integrity and cell death. Table 1 compares the ability of various particles to induce lipid peroxidation *in vitro*. When data are normalized to equivalent surface area, there is a correlation between the pathogenicity of a particle and its ability to induce lipid peroxidation (i.e., silica > crocidolite > talc ≈ bentonite > kaolin).[1,2] Hydroxyl radicals (ȮH) are thought to play an important role in the peroxidation of membrane lipid. The reaction scheme is as follows:

$$\dot{O}H + \text{lipid}(L - H) \rightarrow \dot{L} + H_2O \quad (1)$$

$$\dot{L} + O_2 \rightarrow L\dot{O}O \quad (2)$$

$$L - H + L\dot{O}O \rightarrow \dot{L} + LOOH \quad (3)$$

This scheme indicates that once lipid peroxidation is initiated by hydroxyl radicals, the generation of lipid radicals ($\dot{L}$ and $L\dot{O}O$) can be self-perpetuating.

Particles such as crocidolite contain surface iron, which can, via Fenton-like reactions, generate hydroxyl radicals.[3] The following reactions can occur with crocidolite surface iron:

$$(\text{cysteine on proteins})^n + Fe^{3+} \rightarrow Fe^{2+} + (\text{proteins})^{n+1} \quad (4)$$

$$Fe^{2+} + O_2 \rightarrow Fe^{3+} + O_2^- \quad (5)$$

$$2O_2^- + 2H \rightarrow H_2O_2 \quad (6)$$

$$H_2O_2 + Fe^{2+} \rightarrow \dot{O}H + Fe^{3+} + OH^- \quad (7)$$

Silica, in the form of crystalline SiO_2, can generate surface $\dot{S}i$ and $Si\dot{O}$ upon fracturing, which can be measured by electron spin resonance spectroscopy (ESR).[4] Upon contact with aqueous solutions, these silica-based surface radicals generate hydroxyl radicals which can be identified by a characteristic ESR signal using hydroxyl spin traps.[4] Evidence indicates that the ability of silica to peroxidize lipids is directly related to its ability to generate hydroxyl radicals.[5] Evidence also indicates a strong correlation between the ability of a particle to cause

TABLE 1. Particle-Induced Lipid Peroxidation

Particle	Lipid Peroxidation[A]	Reference
Kaolin	+	1
Bentonite	++	1
Talc	++	1
Crocidolite	+++	2
Silica	++++	1

[A]Normalized to equivalent surface area. Potency is proportional to the number of + signs.

lipid peroxidation and its ability to decrease membrane integrity of lung cells *in vitro*.[1,6]

Recent evidence also indicates that pathogenic particles induce apoptosis (i.e., programmed cell death) in pneumocytes which can be measured as DNA fragmentation *in vitro*.[7,8] As shown in Table 2, particles that are pathogenic (e.g., crystalline silica or asbestos) induce apoptosis, while less pathogenic particles do not.

Activation of Oxidant Release from Phagocytes

Evidence indicates that particles can interact with scavenger receptors on the surface of alveolar macrophages.[8,9] Particle–receptor binding of pathogenic particles, such as crystalline silica, is associated with respiratory burst activity and the production of reactive forms of oxygen, such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical ($\dot{O}H$).[10] The generation of these reactive oxygen species involves a membrane-bound enzyme, NADPH oxidase, as follows:

$$O_2 + NADPH \xrightarrow[\text{Oxidase}]{\text{NADPH}} O_2^- + NADP^+ + H^+ \quad (8)$$

$$2O_2^- + O_2 + 2H^+ \rightarrow H_2O_2 + 2O_2 \quad (9)$$

$$O_2^- + H_2O_2 \rightarrow \dot{O}H + OH^- + O_2 \quad (10)$$

In contrast to silica, less pathogenic particles, such as titanium dioxide or carbonyl iron, cause only slight activation of oxidant release from alveolar macrophages after *in vivo* exposure (Table 3).

Another source of reactive species in pulmonary phagocytes is the cytosolic enzyme, nitric oxide synthase (NOS). Upon exposure to pathogenic particles such as silica, mRNA levels

TABLE 2. Particle-Induced Apoptosis

Particle	Apoptosis
Silica (crystalline)	+
Silica (amorphous)	−
Titanium dioxide	−
Chrysotile	+
Crocidolite	+
Wollastonite	−

Modified from Holian[7] and Iyer *et al.*[8] Positive activity is noted as +, while lack of activity is noted as −.

TABLE 3. Particle-Induced Oxidant Generation

Particle	Chemiluminescence[A]
Silica	680
Carbonyl iron	68
Titanium dioxide	26

[A]Chemiluminescence generated from alveolar macrophages harvested from rats by bronchoalveolar lavage 24 hours after intratracheal instillation of particles (5 mg/100 g body wt). Values normalized to equivalent particle number. Data modified from Blackford *et al.*[11]

for the inducible form of this enzyme (iNOS) become elevated, resulting in the activation of the iNOS enzyme and the production of nitric oxide.[12] As shown by the following reactions, nitric oxide ($N\dot{O}$) can result in the generation of other reactive species [i.e., peroxinitrite ($OONO^-$) and hydroxyl radical ($\dot{O}H$)]:

$$\text{L-arginine} \xrightarrow[\text{synthase}]{\text{NO}} \text{L-citruline} + N\dot{O} \quad (11)$$

$$N\dot{O} + O_2^- \rightarrow OONO^- \quad (12)$$

$$OONO^- + H^+ \rightarrow HOONO \rightarrow \dot{O}H + NO_2 \quad (13)$$

In contrast to silica, less pathogenic particles, such as titanium dioxide or carbonyl iron, cause only slight induction of iNOS message or production of NO by lung phagocytes after *in vivo* exposure (Table 4). Evidence indicates that *in vitro* exposure of lung epithelial cells to crocidolite also results in induction of iNOS message and $N\dot{O}$ production.[13]

Secretion of Inflammatory Cytokines

As described above, pathogenic particles can produce hydroxyl radicals directly or can activate the production of reactive oxygen and/or nitrogen species from alveolar macrophages. It is believed that this excess production of oxidants may cause cleavage of inhibitor proteins and thus activation of the transcription factor, nuclear factor kappa B (NF-kB). Pathogenic particles, such as silica or crocidolite, have been shown to activate NF-kB binding to DNA in lung cells.[14,15] Such NF-kB activation has been associated with secretion of initiating cytokines, such as tumor necrosis factor alpha (TNF-α) and interleukin-1, from alveolar macrophages. TNF-α has been shown to regulate the production of recruitment chemokines such as macrophage inflammatory protein (MIP-1

TABLE 4. Particle-Induced Nitric Oxide Production

Particle	$N\dot{O}$	iNOS Message
Silica	179	27
Carbonyl iron	9	4
Titanium dioxide	6	3

$N\dot{O}$ production and mRNA for iNOS measured in lung phagocytes harvested from rats by bronchoalveolar lavage 24 hours after intratracheal instillation of particles (5 mg/100 g body wt). $N\dot{O}$ production measured as L-NAME inhibitable chemiluminescence. Values normalized to equivalent particle number. Data modified from Blackford *et al.*[11]

TABLE 5. Particle-Induced Production of Inflammatory Cytokines

Particle	NF-kB	TNFα	IL-1	MIP-2	CINC
Silica	+[14]	+[17]	+[17]	+[16]	+[16]
Titanium dioxide	−[18]	−[18]	−[18]	−[18]	−[18]
Crocidolite		+[18]	+[19]	+[16]	+[16]
Chrysotile		+[19]	+[19]		

Positive activity is noted as +, while lack of activity is noted as −. Superscripts denote reference citations.

and MIP-2) and cytokine-induced neutrophil chemoattractant (CINC).[16] These chemokines are potent chemoattractants for neutrophils. Indeed, a direct correlation has been shown between the ability of particles to increase TNF-α production in alveolar macrophages and the recruitment of neutrophils into the air spaces of particle-exposed lungs.[17] Table 5 summarizes the potency of various particles to activate NF-kB and induce the secretion of initiating cytokines and recruitment chemokines.

The result of particle-induced cytokine and chemokine production is the recruitment of neutrophils (PMN) into the airways, as well as activation of those PMN to produce reactive species which would damage lung tissue. Indeed, there is a good correlation between the ability of various particles to recruit PMN and to damage the lung air/blood barrier (Table 6). There is also a good correlation between the pathogenicity of a particle and its ability to increase lavage PMN and protein levels after *in vivo* exposure, with silica being strongly positive while titanium dioxide, carbonyl iron, aluminum oxide, amorphous silica, and iron oxide were less positive.[11,20–23] As mentioned previously, there is a strong correlation between particle-induced TNF-α levels and PMN recruitment.[17] Indeed, there is evidence that pretreatment of rats with antibodies for TNF-α reduces particle-induced production of the neutrophil chemokines, MIP-1 and CINC.[16]

Secretion of Proliferative Factors

Pathogenic particles have also been shown to increase the production of various proliferative factors from alveolar macrophages and/or alveolar epithelial cells. Chrysotile has been shown to enhance the production of platelet-derived growth factor (PDGF) and transforming growth factor-alpha in lung cells.[24,25] Furthermore, fibronectin and alveolar macrophage-derived growth factor production by alveolar macrophage is enhanced after inhalation of silica.[26] These growth factors can enhance the proliferation of interstitial lung fibroblasts and result in fibrosis. Evidence exists that TNF-α can increase the production of growth factors such as PDGF.[27] Indeed, treatment of silica-exposed rats with anti-TNF reduces lung fibrosis.[28]

TABLE 6. Particle-Induced Lung Inflammation and Damage

Particle	PMN	Protein
Silica	116	24
Carbonyl iron	5	10
Titanium dioxide	2	1

Lavage PMN and protein levels 24 hours after intratracheal instillation of particles (5 mg/100 g body wt). Values normalized to equivalent particle number. Data modified from Blackford *et al.*[11]

Conclusion

The relative pathogenicity of particles seems to be related to their ability to enhance the oxidant burden of the lung. Particles can generate oxidants directly, as do crocidolite or fractured silica, or they can activate production of oxidants by pulmonary phagocytes. These oxidants can cleave inhibitor proteins from NF-kB, which results in activation of the binding of this transcription factor to DNA. This DNA binding may induce the production of a host of inflammatory and proliferative factors, which would lead to lung damage and fibrosis. Data to date suggest that TNF-α may play an important role in the stimulation of both inflammatory chemokines and fibrogenic factors.

Several factors seem to be important in determining the relative pathogenicity of a particle. For example, surface radicals and surface iron seem to play a role in the cytotoxicity of a particle. Surface charge seems important, since the scavenger receptor of the alveolar macrophage membrane has an affinity for anionic ligands. Indeed, neutralization of the negative surface charge of silica with aluminum (Al^{3+}) salts has been shown to significantly decrease the *in vitro* cytotoxicity of silica[29] as well as its inflammatory potency *in vivo*.[30] Particle dimension is important, since long, thin fibers result in frustrated phagocytosis and the continued release of reactive products from alveolar macrophages, which may result in increased cytotoxicity.[31]

In summary, it is clear that continued advances in our mechanistic understanding of particle-induced lung disease will be invaluable in predicting the potential pathogenicity of new materials and in determining what exposure limits may be necessary to prevent lung disease from certain materials currently classified as PNOC/R.

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