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International Immunopharmacology 2 (2002) 173–182

International Immunopharmacology

www.elsevier.com/locate/intimp

Review

Diseases caused by silica: mechanisms of injury and disease development

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Abstract

While silica particles are considered to be fibrogenic and carcinogenic agents, the mechanisms responsible are not well understood. This article summarizes literature on silica-induced accelerated silicosis, chronic silicosis, silico-tuberculosis, bronchogenic carcinoma, and immune-mediated diseases. This article also discusses the generation of reactive oxygen species (ROS) that occurs directly from the interaction of silica with aqueous medium and from silica-stimulated cells, the molecular mechanisms of silica-induced lung injuries with focus on silica-induced NF-κB activation, including its mechanisms, possible attenuation and relationship to silica-induced generation of cyclooxygenase II and TNF-α. Silica-induced AP-1 activation, protooncogene expression, and the role of ROS in these processes are also briefly discussed. © 2002 Published by Elsevier Science B.V.

Keywords: Silica; Reactive oxygen species; NF-κB

1. Introduction

Silicosis is one of civilization's oldest known occupational diseases, and is induced by the inhalation of crystalline silica [1,2]. In industrialized countries, the disease is well recognized and is adequately prevented by ambient dust control measures. Nevertheless, overt lung disease caused by the inhalation of silica continues to occur even in developed countries at an alarming rate. The incidence of overt disease is greatly increased in industrial operations by the mechanization and use of sand blasting, drilling, pulverizing, cutting, grinding tools and other pneumatic equipment. Epidemiological and experimental studies have implicated silica not only as causing chronic inflammatory lung disease, but also as a potential carcinogen and the International Agency for Research on Cancer (IARC) concluded that there was sufficient evidence in humans and experimental animals for the carcinogenicity of silica in the forms of quartz and cristobalite [3,4]. Exposure to silica can result in or contribute to many other diseases including acute silicosis, accelerated silicosis, simple silicosis, complicated or conglomerate silicosis, pulmonary tuberculosis, interstitial fibrosis, industrial bronchitis, small airway disease, industrial bronchitis, emphysema, rheumatoid complications, vascular diseases, glomerulonephritis, and immunologic reactions. This review will describe briefly only the salient features of these diseases.

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1567-5769/02/$ - see front matter © 2002 Published by Elsevier Science B.V.
PII: S1567-5769(01)00170-9

2. Diseases caused by silica

2.1. Acute silicosis

Acute silicosis is morphologically manifested by the uniform filling of alveoli by eosinophic-granular, lipid-rich pulmonary edema (silicolipoproteinosis) and interstitial inflammation, characterized by neutrophils and alveolar macrophages containing lamellar bodies. Acute silicosis occurs in workers exposed to mechanically broken or fractured silica particles in occupations such as sand blasting, surface drilling, tunneling, silica flour milling and grinding. The disease can be fatal and often progresses rapidly with severe loss of pulmonary function and difficulty in gas exchange.

2.2. Accelerated silicosis

Accelerated silicosis is morphologically very similar to acute silicosis exhibiting exudative alveolar lipoprotenosis associated with chronic inflammation with the development of rapid and progressive silicosis lesions [2]. In accelerated silicosis, alveolar septa are usually thickened with hypertrophic and hyperplastic type II epithelial cells. An increased number of alveolar macrophages are present with lamellar bodies and silica particles. Similar to acute silicosis, accelerated silicosis is associated with occupations where silica is mechanically fractured or crushed and results in intense exposure.

2.3. Chronic silicosis

Chronic silicosis is the most common form of silicosis and is characterized by the development of distinct, discrete, nodular, whorled lesions in the lung which are hyalinized and frequently contain silica inclusions [2]. These lesions are present more in the upper lobes, and the pleura may be thickened and contain candle-wax lesions [1]. The pulmonary silicotic lesions are usually manifested within 10 or more years of exposure with dust containing 18–30% of crystalline silica. Nodules measure 4 to 10 mm in diameter and with continued exposure, become larger and densely profuse. Variable amounts of dust-laden macrophages and cellular infiltrates are present in peripheral zones. The fully developed silicotic nodule is composed of concentric fibrotic hyalinized laminar layers in the center usually with calcification and minimal amounts of dust. These nodular lesions are very unique pathologic entities in silicosis and can be present in simple or complicated silicosis. Simple silicosis is characterized by lesions less than 1 cm in diameter and usually is not associated with detectable pulmonary functional impairment. With passage of time and continued exposure to silica, these lesions enlarge symmetrically and fuse resulting in the destruction of surrounding pulmonary architecture and producing progressive massive fibrosis or conglomerate silicosis. Complicated silicosis lesions are greater than 1 cm in diameter and are associated with decreased lung function. Necrosis and cavitation of the lesions are common as are accompanying mycobacterial infection.

2.4. Silico-tuberculosis

Workers with silicosis are at an increased risk for developing tuberculosis despite the reduction in prevalence of tuberculosis in the general population [5,6]. It is well documented that exposure to silica can lead to impaired cell-mediated immunity. Alterations in lymphocyte subsets (reduced number of T cells and increased number of B cells) and serum immunoglobulins levels in patients with silicosis have been observed in workers with silicosis [2].

2.5. Bronchogenic carcinoma

IARC recently concluded that there was sufficient evidence to justify that crystalline silica as a human carcinogen [3]. However, interpretation of epidemiologic and experimental evidence is debatable as many epidemiological studies are either negative or equivocal [7], and increased prevalence of cancer can be found in industrial operations irrespective of silica exposure [8]. On the other hand, studies in rats provide compelling evidence that carcinogenicity of silica occurs in a dose-dependent basis. These tumor usually arise in close proximity to fibrous silicosis lesions [9–11]. In other species, such as mice, hamster and guinea pigs, silica has not been found to be carcinogenic.

2.6. Immune-mediated diseases

As will be discussed in detail in a later chapter, autoimmune-like diseases have been linked to silica or

silicosis including rheumatoid arthritis, systemic sclerosis (scleroderma) and systemic lupus erythematosus [1]. Increases in serum polyclonal immunoglobulins associated with increased levels of circulating rheumatoid factor, anti-nuclear antibodies and immune complexes are often associated with some of these conditions. Amorphous silicone and crystalline silica share these common pathogenic responses but little insight into the understanding of the mechanisms has been provided.

3. Silica and generation of reactive oxygen species (ROS)

ROS may be mediators of silica-induced responses including DNA modifications [12,13], inflammation, fibrosis [14], acute cell injury and proliferation [15,16]. Silica can induce ROS generation directly and/or by stimulation of cells.

3.1. Free radical generation directly from silica

During the late 1980s, electron spin resonance (ESR) studies demonstrated the generation of silicon-based free radicals ($Si^{\cdot}$, $SiO^{\cdot}$, $SiOO^{\cdot}$) from freshly fractured silica in air [17,18]. The amount of the free radicals generated decreased with time. These observations indicate that the reactivity of freshly fractured silica may be different from that of aged silica. Measurements on silica-induced toxicity and lipid peroxidation show that freshly fractured silica is more toxic and more potent in inducting lipid peroxidation than aged silica [18]. These studies suggest that free radicals generated directly from silica may be involved in the mechanism of silica-induced cellular injury.

Using ESR spin trapping, it has been shown that freshly fractured silica suspended in aqueous medium is able to generate $^{\cdot}OH$ radical [19–26]. The $^{\cdot}OH$ radical yield increases with prolonged grinding as the number of fractured planes increases. The yield decreases by more than half when the same silica particles are stored in air for 4 days. Catalase, a scavenger of H_2O_2, inhibited the $^{\cdot}OH$ generation, while addition of H_2O_2 enhanced the yield, suggesting that H_2O_2 is a key intermediate in $^{\cdot}OH$ generation. The yield of H_2O_2 generation, depending on the pH and the temperature of hydrolysis, is high enough to be measured by a standard analytical chemistry methods, the MnO_4^- reaction. ESR spin trapping studies also show that freshly fractured silica particles in aqueous media are able to generate superoxide radicals ($O_2^{\cdot -}$). In an aqueous suspension of freshly fractured silica, molecular oxygen is rapidly consumed, which appears to be the source of ROS generation by silica reactions [24].

3.2. Free radical generation from silica-stimulated cells

The generation of ROS represents one of the main mechanisms by which phagocytes kill invading organisms. ROS production increases in response to stimulation and phagocytosis of microorganisms, particulates, and chemicals, resulting in an increase in oxygen consumption called the ‘‘respiratory burst’’. Crystalline silica is a potent stimulant of the respiratory burst in alveolar macrophages [26,27]. The respiratory burst is associated with elevated production of ROS as demonstrated by the following observations: (a) silica stimulates $O_2^{\cdot -}$ production; (b) H_2O_2 release is increased in alveolar macrophages exposed to silica; (c) enhanced chemiluminescence and generation of ROS are observed after in vitro exposure of macrophages to silica. In each of these instances, the ability of silica to affect the respiratory burst is rapid, reaching a maximum within a few minutes following in vitro exposure. ESR spin trapping and chemiluminescence studies have shown that the phagocytosis of silica particles by rat alveolar macrophages and human neutrophils results in enhanced of ROS generation [26,28]. Silica also stimulates the release of chemotactic agents, such as platelet-activating factor (PAF), macrophage inflammatory protein 2 (MIP-2), and cytokine-induced neutrophil chemoattractant (CINC), from alveolar macrophages [29–31]. These factors cause the infiltration of neutrophils into the air space, where macrophage-derived platelet-activating factor and other related factors might stimulate oxygen metabolism, leading to ROS generation. Silica can also be phagocytized by phagocytic cells and is a stimulant of the respiratory burst in alveolar type II epithelial cells in vitro and induces cell signaling events and DNA synthesis via oxidative stress [16].

3.3. Activation of NF-κβ by silica

NF-κβ is a transcription factor that regulates a variety of genes whose products are involved in inflammatory, fibrogenic or acute phase responses, such as cytokines and surface receptors [32–38]. In cells with inducible NF-κB activity, the active form of this factor is composed of two different subunits, p50 and p65 [44]. In resting cells, NF-κB is retained in the cytoplasm in an inactive form and its DNA-binding activity and nuclear/cytoplasmic distribution are controlled by binding to an inhibitory protein known as inhibitor-α (IκBα) and -β of NF-κB, p105 (precursor of p50) and p100 (precursor of p52). Upon activation with extracellular stimuli, these inhibitory proteins are proteolytically degraded or processed by proteasomes and certain proteases, which allows NF-κB to be released and translocate into the nucleus in an activated form. This translocation initiates or regulates early response gene transcription by binding to a decameric motif GGGRNNYYCC (κB elements) found in promoter regions of cellular or viral genes. Recent evidence has indicated that ROS are mediators of NF-κB activation [39,40] and is activated in response to a variety of environmental agents such as heavy metals [41] and asbestos which possess oxidant activities [42,43]. Since NF-κB is an oxidative-sensitive transcription factor, it is possible that signals for a variety of silica-induced responses are due to this signaling component, which is regulated by ROS. Our laboratories have shown that silica activates NF-κB in the mouse macrophage-like cell line, RAW 264.7 cells [45]. While only a p50/p65 or p52/p65 heterodimer could be detected in LPS-stimulated cells, both heterodimers and a p50/p50 homodimer were detected in silica stimulated cells. The silica-induced NF-κB activation was both dose- and time-dependent, with peak activation appearing at a silica dose of 100 μg/ml following incubation for of 12 h. Further studies demonstrated that ROS are involved in silica-induced NF-κB activation. Among ROS, ·OH radicals may play a key role. This is supported by the following observations: (a) silica is able to generate ·OH radical in the presence and absence of H_2O_2 as demonstrated by spin trapping measurements [18,23,26]. (b) Catalase, a scavenger of H_2O_2, blocks NF-κβ activation by silica which SOD, a scavenger of $O_2^{\cdot-}$ and a promoting agent of H_2O_2 generation, exhibits an opposite effect. In this respect, earlier studies have shown that molecular oxygen is consumed to generate $O_2^{\cdot-}$ by silica suspensions [21,46] and silica-induced DNA damage is inhibited in an argon atmosphere, indicating that the role of molecular oxygen in ROS generation [21,46]. It appears that O_2 is reduced to $O_2^{\cdot-}$, which generates H_2O_2. H_2O_2 produces ·OH radical via a Fenton or Fenton-like reaction. The equation for ROS generation is:

$$O_2 \rightarrow O_2^{\cdot-} \rightarrow H_2O_2 \rightarrow {}^{\cdot}OH$$

The metal iron, Fe(II), present on many natural fibers such as asbestos and silica, enhances NF-κβ activation. It is known that Fe(II) generates ·OH from H_2O_2 via the Fenton reaction:

$$Fe(II) + H_2O_2 \rightarrow Fe(III) + OH^- + {}^{\cdot}OH$$

Fe(III), on the other hand, is unable to generate ·OH radical without being first reduced to Fe(II), and finally, (d) the metal chelator, deferoxamine, which reduces NF-κB activation by silica, chelates metal ions, such as Fe(II) or Fe(III), to make them less reactive toward H_2O_2 and, thus, attenuates the generation of ·OH radicals. In contrast, the antioxidant, ascorbate and an ·OH radical scavenger, formate, inhibit NF-κB activation by silica.

3.4. Role of SiOH group in silica-induced NF-κB activation

SiOH groups on the silica surface are thought to be involved in cellular damage [47–50]. Chemical modifications of the silica surface can be used to reduce toxicity in vitro and fibrosis in vivo. It is known that when silica particles are exposed to water, surface silicon–oxygen bonds (Si—O) are hydrated, resulting in the formation of SiOH groups and poly(2-vinylpyridine-*N*-oxide) (PVPNO) is able to bind to SiOH groups. PVPNO has been shown to inhibit silica-induced toxicity, to decrease and delay the development of silicosis in experimental animals and in humans, and to block the interaction of silica surface with phosphate groups of DNA in vitro [51–53]. In this respect, PVPNO inhibits silica-induced production of oxygen radicals in cells [54,55]. Previously, ESR spin trapping measurements have shown the

inhibitory effect of PVPNO on ·OH generation by silica plus H_2O_2 [45], implying that the SiOH group is involved in the generation of ·OH radicals from H_2O_2 by silica. Our laboratories have shown that PVPNO decreases silica-induced NF-κB activation [55]. This inhibition may be due to the combination of the two factors: inhibition of ·OH radical generation by silica; and binding of SiOH groups on the silica surface, resulting in reduction of hydrogen bonding between the silica surface and atomic oxygen on the cell membrane.

Deferoxamine also reduces silica-mediated ·OH radical generation and attenuates silica-induced NF-κB activation. Deferoxamine is widely used for the prevention and treatment of iron overload [56,57] and inhibits ·OH radical generation from H_2O_2 by transition metals [58–60]. High doses of deferoxamine (50 mg/kg/day) can be safely injected into humans [47]. Thus, further investigation on the use of deferoxamine or other metal chelators may offer a possible preventative strategy against silica-induced fibrosis and carcinogenesis.

3.5. Role of NF-κB in silica-induced cyclooxygenase II

Products of arachidonic acid metabolism are critical participants in the development of inflammatory responses after infection or tissue injury. Prostaglandin E_2 (PGE_2) is one of the most studied mediators of this process [61]. The cyclooxygenase (COX) enzyme is considered to be the rate-limiting step for PGE_2 formation. Mammalian cells contain two related isoforms of COX that are encoded by separate genes, referred to as COX I and COX II [62]. The latter is expressed after exposure to mitogenic or pathological stimuli, including cytokines, phorbol esters, and bacterial endotoxin. By virtue of its early induction after stimulation by inflammatory agents, COX II is included in the early response gene family [63]. Although induction of COX II gene expression has been demonstrated, the mechanism responsible remains to be investigated. The NF-κB consensus motifs or similar motifs were found in the COX II genes for nearly all of the species tested [64]. Because NF-κβ plays a key regulatory role in the expression of earlier response genes related to several inflammatory mediators, it is possible that NF-κβ may be important in silica-induced COX II gene transcription. We have shown that suppression of NF-κB activation in alveolar macrophages leads to an attenuation of COX II mRNA accumulation induced by silica [65]. At least two κB sites in the 5′ -flanking region of the rat COX II gene are involved in silica-induced transcriptional control of the COX II gene. The first motif, −404 GGGGATTCCC-395, is absolutely conserved in sequence and is located in a similar position among the COX II genes found in humans, rats, and mice. The second motif, −91 GGGGAAAGCC-82, is conserved only in the mouse and rat COX II genes in sequence and in location. Aspirin, a COX inhibitor, suppressed silica-induced NF-κB activation [60]. PGE_2, one of the downstream reaction products catalyzed by the COX enzyme, was also shown to inhibit silica-induced NF-κB activation by retarding the degradation of NF-κB inhibitor.

It is noteworthy that PGE_2 and COX II inhibitors affect NF-κB activation. Although aspirin can block signal-induced IκBα degradation and subsequent NF-κB activation, the specific target of aspirin appears to be nonspecific. For example, we have found that aspirin may inhibit NF-κB activation via its antioxidant activity [45]. In regard to PGE_2, it has been shown that PGE_2 inhibits immune cell proliferation and downregulates several cytokines, including IL-1, IL-2, and IFNγ [66]. This may be partially linked to its ability to suppress the degradation of IκBα and consequently block the cytoplasmic/nuclear translocation of NF-κB that is essential for transcriptional regulation of cytokine gene expression. In the case of a silica-induced inflammatory gene response, the negative feedback role of PGE_2 on NF-κB activation may be beneficial for host self-protection by tempering the positive feedback loop between the production of cytokines and silica-induced NF-κB activation.

3.6. Role of NF-κB in silica-induced TNF-α production and cytokine expression

Several studies have indicated that macrophage cytokines, mostly notably TNF-α, play an important role in the development of silicosis. Silica is able to stimulate TNF-α release and up-regulate mRNA expression in macrophages [67]. Antisense inhibition of TNF-α mRNA expression causes a corresponding

decrease in silica-induced TNF-α production [68]. The role of TNF-α as a key mediator of silicosis has further been demonstrated by the ability of anti-TNF-α antibodies and exogenous recombinant TNF-α to attenuate and augment, respectively, silicotic fibrosis [69].

The expression of TNF-α is regulated at the transcriptional and translational levels, although it is generally believed that the regulation of TNF-α occurs primarily at the transcriptional level. Several transcription factors including NF-κB, AP-1, AP-2, NFAT, Erg-1, Erg-1, C/EBPβ, Ets, and CREB have been reported to be able to regulate TNF-α gene expression and is dependent upon the nature of the stimulus and the cell type. For example, NF-κB, but not AP-1 or AP-2, is involved in the activation of TNF-α transcription of LPS-stimulated monocytes, whereas NFAT, but not NF-κB, plays a role in PMA-stimulated T cells [70].

We have recently investigated the role of NF-κB and ROS in silica-induced TNF-α production in primary alveolar macrophages and in RAW 264.7 cells [68]. It was demonstrated that inhibition of NF-κB inhibitor by SN50, a specific NF-κB blocker, abolishes silica-induced TNF-α production [71]. Pretreatment of the cells with catalase or deferoxamine, metal chelator to reduce $^{\cdot}OH$ generation, effectively inhibited NF-κB activation and TNF-α production, whereas superoxide dismutase, which converts $O_2^{\cdot -}$ to H_2O_2, has an opposing effect. These results indicate that silica-mediated free radical generation and NF-κB activation may play important roles in silica-induced TNF-α gene expression.

4. Activator protein-1 (AP-1) activation

The activator protein-1 (AP-1) transcription factor plays an important role in cell proliferation, differentiation, inflammation, apoptosis, neoplastic transformation, tumor promotion and metastasis [72–76]. This factor is a complex protein composed of homodimers and heterodimers of the Jun and Fos oncogene families. The genes encoding these protein, c-*jun* and c-*fos*, are inducible by a variety of extracellular stimuli and function as intermediary transcriptional regulators in signal transduction processes leading to proliferation and transformation. The activity of AP-1 is modulated by several factors, including the redox state of the cell. Recent studies have shown that freshly fractured crystalline silica is able to stimulate AP-1 DNA binding activity as well as AP-1 transactivation activity [77,78]. Silica induced an 8-fold increase in AP-1 activity in JB6 cells and a 2.5-fold increase in RLE cells. These studies also demonstrated that freshly fractured crystalline silica stimulates mitogen-activated protein kinase (MAPK) family members, as determined by the phosphorylation of p38 MAPK and extracellular signal-regulated protein kinases (ERKs). Silica also stimulated AP-1 transactivation in pulmonary tissues of AP-1 luciferase reporter mice. At 3 days after intratracheal instillation of silica, AP-1 activity was elevated 23-fold in lung homogenates when compared to control mice.

With regard to the mechanisms of silica-induced AP-1 activation, results show that silica-induced AP-1 activation involves ROS-mediated reactions [16,78]. A major role of H_2O_2 in silica-induced AP-1 activation in JB6 cells [78] is supported by the following observations: (a) catalase, which function is to remove H_2O_2, blocked AP-1 activation and phosphorylation of MAPKs, (b) SOD, which converts $O_2^{\cdot -}$ to H_2O_2, enhanced AP-1 activation and (c) sodium formate, a scavenger of $^{\cdot}OH$ radical, did not exhibit any effect. It is well known that stress-related cellular signals, such as UV radiation or ROS, induce the activation of MAP kinase pathways. ERKs, JNKs, and p38 kinases are important signal transduction pathways for AP-1 activation. Freshly fractured silica has been showed to phosphorylate both p38 kinase and ERKs at higher levels than that induced by aged silica. Moreover, catalase inhibited the silica-induced phosphorylation of ERKs and p38 kinase, showing that H_2O_2 is required in the phosphorylation process. These results further show that H_2O_2 plays a key role in AP-1 activation.

Recently, we demonstrated that silica activates the JNK cascade in C10 murine pulmonary epithelial cells [16]. Exposure to a-quartz silica caused persistent increases in phosphorylated JNKs, and AP-1 activity that were not observed with glass beads (particle control) or when pretreated with catalase or the hydroxyl scavenger, tetramethylthiourea (TMTU). This increased activity was accompanied by mRNA increases in the AP-1 family member, *fra*-1, and its presence in AP-1 complexes. Moreover, silica-exposed

cells exhibited dose-dependent increases in the percentages of cells in S and G2/M phases at lower concentrations and increases in subGo/G1cells, which reflects apoptosis, at higher concentrations. The addition of TMTU selectively inhibited both the increase in *fra*-1 and the percentage of cells in S phase by silica suggesting a role for ROS in expression of AP-1 family members that may be linked causally to DNA synthesis.

Taken together, these data provide a working hypothesis that explains the events responsible for cell proliferation and carcinogenesis by crystalline silica. By activating the AP-1 transcription factor through MAPK signal transduction pathways, silica stimulates chronic cell proliferation, which subsequently contributes to silicosis and carcinogenesis in the lung. It is possible that activation of AP-1 is a crucial event that initiates cell proliferation and progression through the cell cycle. Biopersistent silica particles may provide a prolonged redox signal and growth stimulus during the long latency period of tumorigenesis and thereby contribute to the eventual fixation of genetic changes caused by silica [16,77,78]. Furthermore, the induction of AP-1 activity may affect changes in cell phenotype that contribute to neoplastic transformation.

5. Gene polymorphisms in silicosis

Cytokines play key roles in inflammation, fibrosis, immune responsiveness and development of diseases. A delicate balance between the levels of several cytokines, their receptors and specific inhibitory mechanisms help govern many physiologic and pathologic reactions. Among the various cytokines, IL-1 and TNF-α share many biologic properties and both are produced by activated macrophages. Their secretions can be stimulated by a variety of chemical, physical and biologic factors which in turn can stimulate the release of several chemical mediators, other cytokines, enzymes and a spectrum of changes leading to gene transcription and endothelial activation. In a recent study of coal miners with characteristic pathologic nodular silicosis lesions, the association of TNF-α and IL-1 gene polymorphisms were investigated in 325 autopsied coal miners with moderate and severe silicosis and compared with 164 miners with no lung disease [79,80]. In coal miners with severe silicosis, the TNF-α variant -238 was markedly elevated, while significantly lower for miners with moderate silicosis. However, in all with silicosis the TNF-α (-308) and the Il-1RA (-208) variants were elevated regardless of disease severity. In multigene comparisons, these gene–gene and gene–gene–environment interactions suggest that inflammatory cytokine polymorphisms modify the development and severity of silicosis.

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