Free Radical Biology & Medicine, Vol. 37, No. 7, pp. 916–925, 2004

0891-5849/$-see front matter

ELSEVIER

doi:10.1016/j.freeradbiomed.2004.05.032

Serial Review: Signaling by Toxicants
Serial Review Editor: Henry J. Forman

SIGNALING PATHWAYS CONTROLLING THE PRODUCTION OF INFLAMMATORY MEDIATORS IN RESPONSE TO CRYSTALLINE SILICA EXPOSURE: ROLE OF REACTIVE OXYGEN/NITROGEN SPECIES

VINCENT CASTRANOVA

National Institute for Occupational Safety and Health, Morgantown, WV 26505, USA

(*Received* 7 *November* 2003; *Revised* 7 *May* 2004; *Accepted* 28 *May* 2004)
Available online 20 June 2004

Abstract—Occupational exposure to crystalline silica has been linked to pulmonary fibrosis and lung cancer. Surface properties of crystalline silica are critical to the production of oxidant species, chemokines, inflammatory cytokines, and proliferative factors involved in the initiation and progression of silica-induced damage, inflammation, alveolar type II cell hyperplasia, fibroblast activation, and disease. The transcription factors nuclear factor κB (NF-κB) and activator protein 1 (AP-1) have been shown to play key roles in gene promotion for inflammatory mediators, oncogenes, and growth factors. This review summarizes evidence that in vitro and in vivo exposure to crystalline silica results in activation of NF-κB and AP-1. Signaling pathways for activation of these transcription factors are described. In addition, the role of silica-induced reactive oxygen species and nitric oxide in the activation of these signaling events is presented. Last, the generalizability of mechanisms regulating silica-induced pulmonary responses to pulmonary reactions to other occupational particles is discussed.

Keywords—Silica, Transcription factors, Signaling pathways, Reactive oxygen species, Fibers, Free radicals

Contents

This article is part of a series of reviews on "Signaling by Toxicants." The full list of papers may be found on the home page of the journal.

Address correspondence to: Vincent Castranova, Pathology and Physiology Research Branch, Health Effects Laboratory Division, National Institute for Occupational Safety and Health, MS L-2015, 1095 Willowdale Road, Morgantown, WV 26505, USA.

INTRODUCTION

Inhalation of crystalline silica has long been associated with lung disease [1]. The rate of disease progression, i.e., acute, accelerated, or chronic silicosis, seems to depend on the rate of deposition as well as the total burden of crystalline silica in the lung [2]. Acute silicosis results from exposure to high levels of freshly fractured dust associated with sand blasting, rock drilling, and silica flour milling. Acute silicosis is characterized by a rapid onset (after a few years of exposure) of alveolar proteinosis, edema, and inflammation, which result in a dramatic decrease in gas exchange. Accelerated silicosis develops more slowly and is characterized by alveolar lipoproteinosis, chronic inflammation, and fibrotic granulomas. Chronic silicosis has an onset of 20–40 years and is a restrictive lung disease. It is characterized by silicotic nodules, which are fibrotic lesions of collagen material arranged in a spiral pattern. It has been proposed that silicosis results from a cycle of cell damage, oxidant generation, inflammation, scarring, and fibrosis [2–6]. This scheme involves direct cytotoxicity of crystalline silica on lung cells due to the unique surface properties of quartz. In addition, alveolar macrophages and/or alveolar epithelial cells are stimulated to produce inflammatory chemokines and cytokines. As a result of the recruitment and activation of alveolar macrophages and polymorphonuclear leukocytes, elevated production of reactive oxygen species (ROS) would cause oxidant injury to the lung parenchyma. In response to this oxidant stress and lung injury, alveolar macrophages and/or alveolar epithelial cells would be stimulated to produce growth factors and fibrogenic mediators, resulting in fibroblast activation and pulmonary fibrosis [2–7]. A mechanistic scheme for the pathogenesis of silicosis is given in Fig. 1. The temporal relationship among silica-induced oxidant generation, cytokine production, and pulmonary fibrosis has been described in a 6-month inhalation study by Castranova et al. [8].

In addition to silicosis, inhalation of crystalline silica has been associated with the development of lung cancer [9]. Proposed mechanisms involved in silica-induced carcinogenesis include direct DNA damage, inhibition of p53, loss of cell cycle regulation, stimulation of growth factors, and production of oncogenes [10]. Hyperplasia of alveolar type II epithelial cells has been viewed as a precursor to metaplasia and tumor formation in rat models of particle-induced lung cancer [11].

IMPORTANCE OF SURFACE PROPERTIES OF CRYSTALLINE SILICA

Crystalline silica is silicon dioxide (SiO_2) arranged in a three-dimensional tetrahedral crystal lattice [12]. As such, silanol groups are present on the silica surface. These silanol groups can act as hydrogen donors and form hydrogen bonds with oxygen and nitrogen groups in biological membranes [13]. This close interaction between silica and cell membranes results in loss of membrane integrity, lysosomal enzyme leakage, tissue injury, and lung scarring. Agents which act as hydrogen acceptors, such as polyvinylpyridine-*N*-oxide or organo-

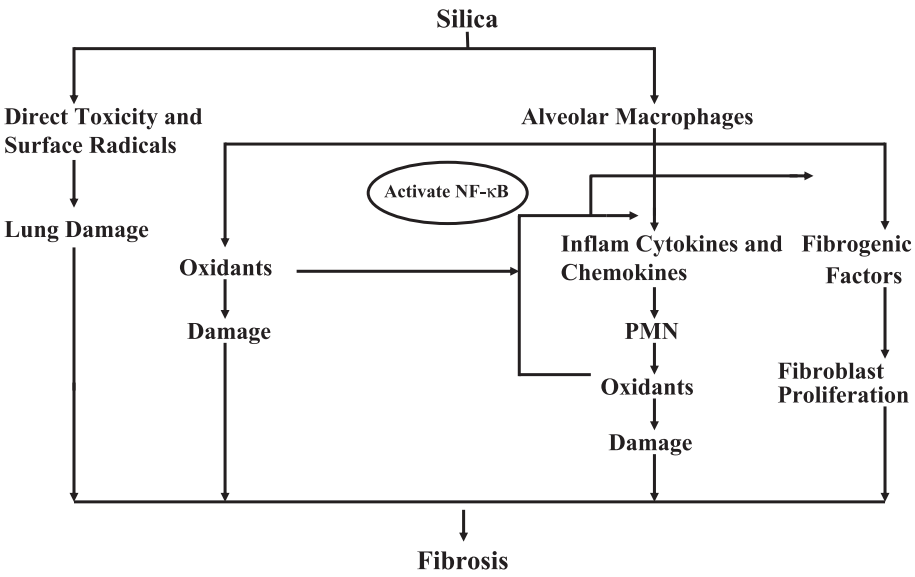

Fig. 1. Proposed mechanisms involved in the initiation and progression of silicosis. Oxidants are generated directly from the reactive surface of crystalline silica and as a result of phagocytosis of silica particles. These reactive oxygen species activate transcription factors controlling message levels in macrophages which induce the production of chemokines, inflammatory cytokines, and fibrogenic factors.

silanes, coat the silica surface and effectively decrease the toxicity of silica in both in vitro and in vivo models [14,15].

At pH 7.0, 1 in 30 surface silanol groups have lost a H^+ to form a SiO^- group [16]. Therefore, silica particles have a negative surface charge [17]. Evidence indicates that this negative surface charge is a significant contributor to the toxicity of silica and that the ζ potential of silica can be reduced to OmV with $AlC1_3$ [16]. Aluminum salts have been reported to reduce the toxicity of silica in both in vitro [16] and in vivo models [18].

Cutting, fracturing, or grinding crystalline silica would cleave Si–O bonds in the crystal lattice and generate siloxyl radicals ($Si^\bullet$ or $SiO^\bullet$). The existence of these siloxyl radicals on the cleavage planes of freshly fractured silica has been demonstrated by electron spin resonance (ESR) spectroscopy [5,19,20]. In aqueous medium, these siloxyl radicals can generate hydroxyl radicals ($^\bullet OH$) [19]. This $^\bullet OH$ generation can be inhibited by metal chelators, suggesting that trace surface iron, which can participate in a Fenton reaction, plays an important role in $^\bullet OH$ generation. As fractured silica is aged in air, surface radicals and the ability to generate $^\bullet OH$ decrease with a $T_{1/2}$ of 30 and 24 h, respectively. Freshly ground silica has been shown to be more cytotoxic to lung cells in vitro (lipid peroxidation and membrane damage) and more inflammatory and pathogenic in vivo than aged silica [21–23]. This enhanced potency of fresh silica has been correlated to the ability to generate radicals. Although radical generation by crystalline silica decreases with time after fracturing, aged silica still generates radicals at a lower level and still exhibits substantial toxicity. This radical generation has been attributed to the Fenton reaction between trace iron and H_2O_2 generated during phagocytosis of silica particles by alveolar macrophages. It is of interest that this active iron seems to be complexed in the crystal lattice of silica rather than easily soluble [24,25].

TRANSCRIPTION FACTOR CONTROL OF INFLAMMATORY MEDIATORS AND GROWTH FACTORS

Exposure to crystalline silica induces the production of chemokines, inflammatory cytokines, and growth factors from alveolar macrophages and alveolar type II cells, which have been linked to the initiation and progression of silica-induced lung disease [2–7]. Tumor necrosis factor-α (TNF-α) has been proposed as a critical mediator in the pathogenesis of silicosis [2]. Driscoll and colleagues have shown a direct relationship between silica-induced production of TNF-α by alveolar macrophages and infiltration of inflammatory cells into the lungs of silica-exposed rats [26]. This TNF-α dependent inflammation has been linked to the production of macrophage inflammatory protein-2 (MIP-2), i.e., a chemokine for neutrophils. In addition, treatment of mice with an antibody to TNF-α has been shown to decrease MIP-2 production, inflammation, and the resulting pulmonary fibrosis [2,27]. Interleukin-1 (IL-1) has also been shown to affect pulmonary inflammation by stimulating the production of chemokines and the expression of adhesion molecules [26]. Recent data indicate that the number and size of silica-induced granulomas are dramatically decreased in IL-1 knockout mice compared to wild-type mice at 1, 6, and 12 weeks postexposure [28]. IL-1 knockout mice also exhibited lower levels of inducible nitric oxide synthase (iNOS) expression and apoptosis in silica-exposed lungs compared to wild-type mice.

NF-κB is a transcription factor that controls the gene expression of a variety of chemokines, cytokines, adhesion molecules, and growth factors [29]. Activation of NF-κB has been linked to the induction of a number of chronic inflammatory diseases [30]. NF-κB is a dimer, with the most abundant form being a heterodimer of p50 and p65 subunits. In resting cells, NF-κB resides in the cytoplasm in an inactive form bound to an inhibitory protein (IκB). Upon activation of the cell, IκB is phosphorylated by IκB kinase and then ubiquitinated, allowing dissociation of IκB from NF-κB. This active NF-κB can then translocate into the nucleus and bind to specific sites on the gene promoter for various mediators. NF-κB/DNA binding activates transcription of mRNA for these mediators, which induces translational production of the specific chemokine, cytokine, or growth factor. The free IκB is in turn degraded by proteasomes. This process is depicted in Fig. 2.

AP-1 is composed of homo- or heterodimers of the protein products of individual members within the Jun (c-Jun, c-Jun-B, and c-Jun D) and Fos (c-Fos, FOS B,

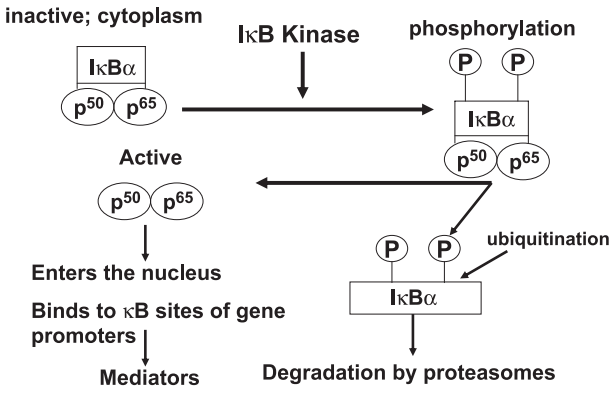

Fig. 2. Pathways for NF-κB activation. Phosphorylation of IκB-α results in dissociation of this inhibitor from the NF-κB complex. This active NF-κB can then translocate from the cytoplasm to the nucleus, bind to gene promoter sites, and produce mRNA controlling various inflammatory cytokines and growth factors.

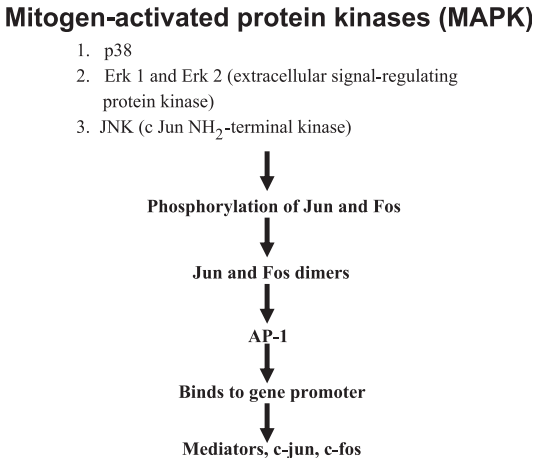

Fig. 3. Pathways for AP-1 activation. MAP kinases regulate phosphorylation of Jun and Fos, which form activated AP-1 that binds to gene promoter sites. AP-1 activation controls the production of inflammatory mediators, growth factors, and oncogenes.

Fra-1, and Fra-2) immediate-early response gene families. AP-1 can bind to the TPA response elements in the promotor or 5′ flanking regions of a number of target genes, such as those encoding c-jun, c-fos, Egr 1, Egr 2, collagenase, stromelysin, certain cytokines, cyclin D, transforming growth factor-β, and a number of other genes involved in both inflammation and carcinogenesis [31–34]. The activation pathway for AP-1 is shown in Fig. 3. It involves activation of mitogen-activated protein kinases and subsequent phosphorylation of Jun and Fos, which dimerize to form AP-1 [31–34].

SILICA-INDUCED ACTIVATION OF NF-κB

In vitro exposure of mouse peritoneal macrophages (RAW 264.7 cells) to silica caused a significant enhancement of the DNA-binding activity of NF-κB, measured by the electrophoretic mobility shift assay [35–37]. After a 4 h exposure, 25 μg/ml silica increased NF-κB activity by 250%, with maximal activation (650%) occurring after exposure to 100 μg/ml silica [36]. Such silica-induced activation of NF-κB in RAW 264.7 cells has been linked with the production of TNF-α, a proinflammatory and fibrotic cytokine [38,39]. An initial response of this macrophage cell line to silica exposure (100 μg/ml) was the generation of reactive oxidant species, measured as chemiluminescence after a 10 min exposure. Under these conditions, such exposure increased oxidant production by these phagocytes to 200% of the control level [36]. This silica-induced oxidant production was effectively inhibited by superoxide dismutase (SOD; 615 U/ml) or catalase (3750 U/ml). A 2 h pretreatment of macrophages with antioxidants, i.e., SOD, catalase, vitamin E, pyrrolidine dithiocarbamate (PDTC), or *N*-acetylcysteine (NAC), also significantly inhibited the ability of silica to activate NF-κB, i.e., inhibition of approximately 50% with SOD or catalase and over 95% with PDTC or NAC [36]. These authors concluded that reactive oxygen species produced by macrophages during phagocytosis of silica act to stimulate signaling pathways for NF-κB activation. Chen et al. [37] have also reported the oxidant dependence of silica-induced NF-κB activation, with catalase, ascorbate, and formate being potent inhibitors of NF-κB activation. They have also shown that silica plus H_2O_2 generated $^{\bullet}OH$ and that a chelator (deferoxamine) decreased the generation of $^{\bullet}OH$ by 76%, measured by ESR. Formate, a $^{\bullet}OH$ scavenger, decreased the ESR signal by 85%, whereas the H_2O_2 scavenger catalase also effectively decreased the ESR signal. Deferoxamine, like formate or catalase, effectively inhibited silica-induced NF-κB activation. These authors, therefore, proposed that $^{\bullet}OH$, generated by a Fenton reaction on the silica surface, played an important role in silica-induced NF-κB activation.

Silica has also been shown to activate NF-κB in primary rat alveolar macrophages as well as RAW cells after a 6 h in vitro exposure [40]. Such silica exposure also resulted in enhanced (420% of control) production of TNF-α by both the primary and the cell line macrophages. Treatment of cells with SN50, an inhibitor of NF-κB translocation to the nucleus, decreased NF-κB/DNA binding, NF-κB activation of the TNF-α gene promotor, and TNF-α production [40]. These authors confirmed the involvement of surface iron and generation of reactive oxygen species in these responses, because catalase or deferoxamine inhibited NF-κB activation, TNF-α gene promotor activity, and TNF-α production in this macrophage system.

Silica-induced activation of NF-κB has also been reported in other pneumocytes, such as Rat 2 fibroblasts [41]; human bronchial epithelial cells, BET-1A [42]; and rat alveolar type II epithelial cells, RLE-6TN [43]. This NF-κB activation has been linked to the production of interleukin 8 [42] and MIP-2 [43] in response to silica exposure. Such silica-induced cell activation was inhibited by the antioxidant *N*-acetylcysteine [42] or by inhibition of mitochrondrial-derived H_2O_2 [43].

Sacks et al. [44] have reported that intratracheal instillation of rats with silica (200 mg/kg) increased NF-κB/DNA binding in bronchoalveolar lavage cells. NF-κB activation was significantly increased as soon as 1 h postexposure and was maintained through 18 h postexposure. Treatment of the rats with dexamethasone, a corticosteroid reported to block NF-κB at the gene promotor or to prevent IκB degradation, decreased NF-κB activity and reduced both phagocyte infiltration into the alveoli and oxidant production by these phagocytes. Porter et al. [45] confirmed this silica-induced NF-κB

activation in an inhalation exposure model. Exposure of rats to 15 mg/m^3 silica for 6 h/d increased the NF-κB activity of bronchoalveolar lavage cells by 200% after 5 d of exposure. This activation rose linearly with increasing duration of exposure, i.e., increasing lung burden, to 460% of control after 116 d of exposure. Data indicate that this NF-κB activation was associated with an exposure-dependent increase in TNF-α and IL-1 production by these cells [45]. Association between silica-induced induction of NF-κB, neutrophil recruitment, and MIP-2 has been reported after intratracheal instillation of silica into rats [46]. Silica-induced NF-κB activation has also been reported in a mouse model [47,48].

Data presented above suggest that reactive oxygen species activate signaling pathways for NF-κB. Kang et al. [49] reported that nitric oxide ($NO^{\bullet}$) may also play an important role in silica-induced NF-κB activation. Using RAW 264.7 macrophages, they reported that silica (100 μg/ml) effectively stimulated $NO^{\bullet}$ production by these phagocytes by 11.7-fold after a 24 h exposure in vitro. This $NO^{\bullet}$ generation was completely inhibited by the nitric oxide synthase inhibitor L-NIL (5×10^{-5} M) and was inhibited up to 86% by L-NAME (1×10^{-3} M). Both L-NIL and L-NAME inhibited silica-induced NF-κB activation in this macrophage cell line up to 92 and 54%, respectively. Incubation of macrophages with $NO^{\bullet}$-generating compounds, such as SNP or SIN-1, increased NF-κB by 340 and 580%, respectively [49]. If $NO^{\bullet}$ plays a role in NF-κB signaling and, as shown above, NF-κB activation results in TNF-α production, one would expect deletion of the iNOS gene in knockout mice to result in a depression of silica-induced TNF-α production in iNOS knockout mice. This was indeed the case as reported by Zeidler et al. [50]. Aspiration of silica (40 mg/kg) in wild-type mice resulted in a 120% increase in TNF-α levels in acellular bronchoalveolar lavage fluid 42 d postexposure. In contrast, lavage TNF-α levels increased after silica exposure by only 23% in iNOS knockout mice. Knockout of iNOS also resulted in a substantial decrease in histological evidence of silica-induced pulmonary damage and inflammation, supporting a role for NO in the pathogenesis of silicosis [28,50].

SIGNALING PATHWAYS FOR SILICA-INDUCED ACTIVATION OF NF-κB

In vitro exposure of RAW macrophages to silica (100 mg/ml for 30 min) resulted in the phosphorylation of a number of cellular proteins with approximate molecular weights of 39, 58–70, and 103 kDa [36]. Pretreatment with a protein tyrosine kinase inhibitor, genistein (74 μM; 2 h pretreatment), completely blocked this silica-dependent protein phosphorylation. Protein tyrosine kinase (PTK) inhibitors, such as genistein, AG-494, and herbimycin A, inhibited silica-induced NF-κB activation by 85, 89, and 94%, respectively. In contrast, protein kinase A or C inhibitors were ineffective. Therefore, Kang et al. [36] proposed the involvement of PTK in the silica-dependent signaling pathway for NF-κB. Western blot analysis indicated that IκB-α is phosphorylated after treatment of RAW cells with silica (100 μg/ml). This response is rapid, occurring within 5 min of exposure [51]. A 2 h pretreatment with PTK inhibitor, genistein (74 μM) or AG126 (30 μM), completely blocked the phosphorylation of IκB. Both silica-induced phosphorylation of IκB and activation of NF-κB were inhibited by antioxidants, such as SOD, NAC, and PDTC. Therefore, Kang et al. [51] concluded that silica-induced oxidants activate tyrosine phosphorylation of IκB-α by PTK, which results in NF-κB activation.

In a recent study, Kang et al. [52] reported that silica induced phosphoinositide 3-kinase (PI3-kinase) activity in RAW macrophages, measured as phosphorylation of phosphatidylinositol to phosphatidylinositol phosphate. This silica-induced activation of PI3-kinase was rapid, significantly increasing within 10 min, and was blocked by wortmannin or LY294003. PI3-kinase inhibitors wortmannin and LY294003 also inhibited silica-induced NF-κB activation by 50 and 70%, respectively. This PI3-kinase-dependent NF-κB activation was mediated through two signaling pathways, i.e., the p110 subunit of PI3-kinase induces the tyrosine phosphorylation of the p65 monomer of NF-κB, which is believed to enhance DNA binding, and the p85α subunit of PI3-kinase binds to IκB-α. However, the PI3-kinase effect on NF-κB activation did not involve the tyrosine phosphorylation of IκB-α, which was unaffected by wortmannin [52]. The silica-induced activation of PI3-kinase seems to involve reactive oxygen species, because SOD, NAC, and PDTC inhibit PI3-kinase as well as NF-κB activity [52].

Figure 4 summarizes the current information concerning signaling pathways involved in silica-induced production of chemokines, inflammatory mediators, and growth factors. Activation of NF-κB is key to gene expression, induction of mRNA, and production of mediators critical for disease initiation and progression. PTK and PI3-kinase control key phosphorylation steps in the activation of NF-κB. Oxidant species derived directly from the silica surface or indirectly as part of the phagocytotic process act to trigger signaling pathways controlled by both PTK and PI3-kinase.

SILICA-INDUCED ACTIVATION OF AP-1

AP-1 activity can be measured in cell lines transfected with an AP-1 luciferase reporter plasmid which generates light upon complexation of AP-1 with its DNA binding sites. In vitro exposure of rat lung type

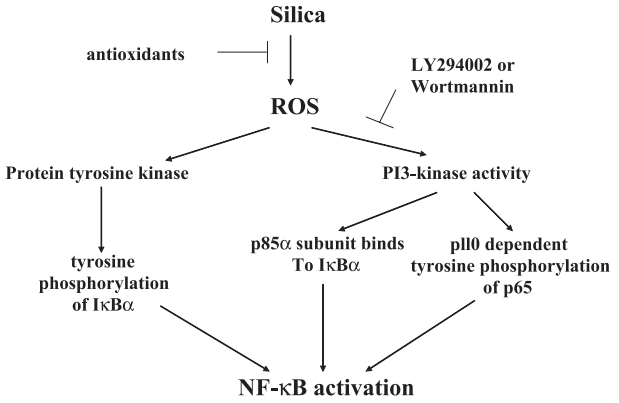

Fig. 4. Silica-induced signaling pathway for NF-κB. Silica-induced reactive oxygen species activate PTK and PI3-kinase, which induce steps critical to inactivation of IκB-α and activation of NF-κB.

II epithelial cells (RLE-6TN) or mouse epidermal cells (JB6 P^+) to 200 μg/ml silica for 36 h increased AP-1 activity by 1.8- or 6.3-fold, respectively [53]. Silica induction of AP-1 activity has been reported for macrophages and alveolar type II cells by other labs [32,38]. Intratracheal instillation of AP-1 luciferase transgenic mice with 5 mg of silica increased AP-1 activity of lung tissue 5.5- and 16.5-fold at 2 and 3 d postexposure, respectively [53]. These in vivo results are supported by studies from other labs as well [48,54]. Evidence suggests that oxidants play a role in this silica-induced activation of AP-1. For example, a suspension of JB6 P^+ cells and freshly fractured silica generated more $^{\bullet}OH$ in aqueous medium than aged silica [55]. A Fenton reaction seems to be involved, since this $^{\bullet}OH$ production was effectively decreased by the H_2O_2 scavenger catalase (64% inhibition) and the iron chelator deferoxamine (46% inhibition). Freshly fractured silica also stimulated more cell-associated H_2O_2 and $O_2^{\bullet}$ than aged silica. Ding et al. [55] have shown that fresh fractured silica is 4.75-fold more potent in activating AP-1 in JB6 P^+ cells than fractured silica aged in air for 1 year. In addition, treatment of JB6 P^+ cells with catalase or deferoxamine decreased silica-induced AP-1 activation by 88 or 25%, respectively [55]. Silica-induced AP-1 activation in alveolar epithelial cells has also been shown to be inhibited by antioxidant treatment [32].

SIGNALING PATHWAYS FOR SILICA-INDUCED ACTIVATION OF AP-1

Ding et al. [53] have shown that silica induced the phosphorylation of mitogen-activated protein kinase

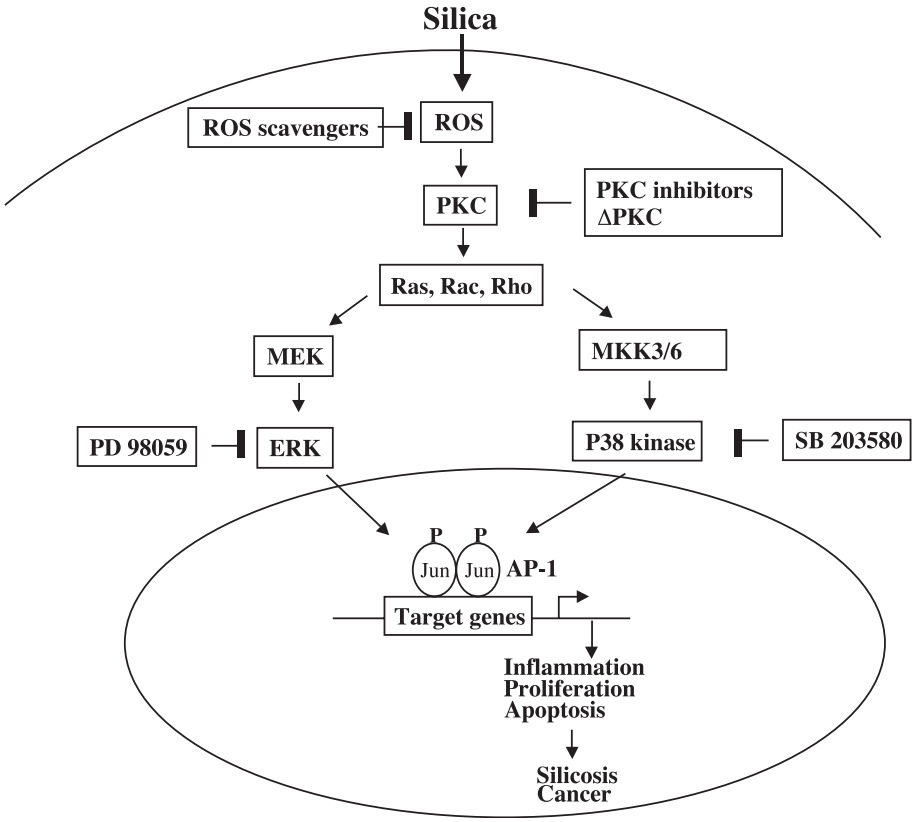

Fig. 5. Silica-induced signaling pathway for AP-1. Silica-induced reactive oxidant species stimulate PKC, which in turn activates MAP kinases and AP-1.

(MAPK) family members, extracellular signal-regulated protein kinases (ERK1 and ERK2), and p38 but not Jun N-terminal kinases in mouse epidermal cells. This MAPK phosphorylation was rapid (first occurring in 15 min and peaking within 2 h of silica exposure) and preceded the activation of AP-1. In addition, inhibition of ERKs or p38 with PD98059 or SB203580, respectively, effectively inhibited silica-induced AP-1 activation in JB6 P^+ cells [53]. Therefore, the authors concluded that ERKs and p38 play an important role in the signaling pathway for silica-stimulated AP-1 activation in this cell type. Silica-induced phosphorylation of ERK was also reported in macrophage and fibroblast cell lines [38,56]. In contrast, silica-induced phosphorylation of JNK was reported in an alveolar type II cell line [32]. Exposure of JB6 P^+ cells to freshly fractured silica caused greater phosphorylation of ERKs and p38 than exposure to aged silica [55]. In addition, catalase was a potent inhibitor of MAPK activation [55,56]. Therefore, the role of oxidants in silica-induced AP-1 activation seems to be mediated through MAPK-dependent pathways.

Exposure of JB6 P^+ cells to silica caused translocation of protein kinase C (PKC) from the cytosol to the membrane within 30 min of exposure. Treatment of cells with inhibitors of PKC effectively decreased silica-induced phosphorylation of ERKs and p38 as well as inhibiting silica-induced AP-1 activation [57]. Therefore, the authors suggested that PKC activation was an early event in the signaling pathway for silica-induced AP-1 activation.

Figure 5 summarizes the current information concerning signaling pathways involved in silica-induced production of inflammatory and growth factors as well as mediators which regulate the cell cycle and proliferation. Activation of the transcription factor AP-1 has been reported to be involved in neoplastic transformation, tumor progression, and metastasis [58–61]. Therefore, AP-1 has been proposed to play a role in silica-induced carcinogenesis [10]. Reactive oxygen species appear to signal phosphorylation of MAPK pathways in a PKC-dependent fashion.

GENERALIZABILITY OF SILICA-INDUCED SIGNALING PATHWAYS TO PULMONARY REACTIONS TO OTHER PATHOGENIC OCCUPATIONAL DUSTS

As with crystalline silica, exposure to asbestos is associated with pulmonary fibrosis and cancer [62]. In many respects, mechanisms for disease development with fibers parallel those proposed for silica. For example, asbestos has been shown to generate reactive oxygen species both by a surface Fenton reaction and during phagocytosis of these fibers [63,64]. These reactive species have been associated with fiber-induced DNA damage, the production of inflammatory cytokines and growth factors, and oncogene expression [65–68].

As with silica, asbestos has been shown to induce NF-κB activation in tracheal epithelial cells, fibroblasts, macrophages, and alveolar epithelial cells [69–74]. This NF-κB activation has been linked with fiber-induced production of inflammatory cytokines and chemokines, such as TNF-α, IL-6, IL-8, and MIP-2 [69,74–76]. Reactive oxygen species have been shown to play an important signaling role in fiber-induced NF-κB activation and resulting cytokine production [70–75].

Asbestos has also been shown to induce AP-1 activation both in cultured cells in vitro and in pulmonary and bronchial tissue after in vivo exposure [77–82]. Asbestos-induced AP-1 activation was preceded by phosphorylation of ERKs. Inhibition of ERKs with PD98059 inhibited fiber-induced AP-1 activation as well as TNF-α production by rat alveolar macrophages [83]. Again, reactive oxygen species have been reported to play a role in the signaling pathway for fiber-induced AP-1 activation [68,77]. Last, as with silica-induced AP-1 activation, asbestos-induced AP-1 activation was shown to be dependent on PKC [84].

These data indicate that striking similarities exist in signaling pathways for NF-κB and AP-1 induction between silica and asbestos exposure models. Therefore, these signaling pathways may be relevant to mechanisms involved in the pulmonary response to a variety of particles which act by generating reactive oxygen species. Indeed, activation of NF-κB has been reported in alveolar macrophages exposed to soot, toner, or TiO_2 treated with iron, and reactive oxygen species have been implicated in the signaling pathway [70,85,86].

SUMMARY

Production of chemokines, inflammatory cytokines, and growth factors is believed to be a key event in the initiation and progression of silica-induced lung disease [2–7]. Transcriptional control of mRNAs for these mediators involves activation of NF-κB and AP-1. Protein tyrosine kinase and phosphatidylinositide 3-kinase have been shown to be critical stimulants of the signaling pathway for NF-κB, whereas protein kinase C and mitogen-activated protein kinases are involved in the signaling pathway for AP-1. Reactive oxygen species, generated directly from the silica surface and/or during phagocytosis of these particles, seem to trigger critical signaling events for both NF-κB and AP-1 activation [5,31]. Therefore, oxidative stress has been proposed to play a key role in the pathogenesis of silicosis and silica-induced lung cancer. Silica-induced signaling mechanisms seem to be models for understanding signaling

pathways for the initiation of pathogenesis in response to other occupational particles.

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