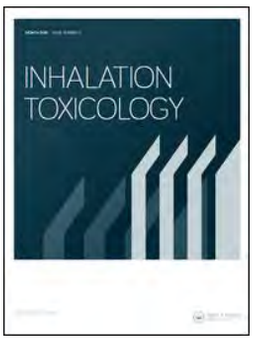

Inhalation Toxicology

International Forum for Respiratory Research

ISSN: 0895-8378 (Print) 1091-7691 (Online) Journal homepage: https://www.tandfonline.com/loi/iiht20

Molecular Activation of Activator Protein-1 In Silica and Asbestos-Induced Carcinogenesis

Val Vallyathan, Min Ding, Xianglin Shi & Vincent Castranova

To cite this article: Val Vallyathan, Min Ding, Xianglin Shi & Vincent Castranova (2000) Molecular Activation of Activator Protein-1 In Silica and Asbestos-Induced Carcinogenesis, Inhalation Toxicology, 12:sup3, 353-357, DOI: 10.1080/08958378.2000.11463245

To link to this article: https://doi.org/10.1080/08958378.2000.11463245

Inhalation Toxicology, 12(Supplement 3):353–357, 2000
0895-8378/00 $12.00 + .00

MOLECULAR ACTIVATION OF ACTIVATOR PROTEIN-1 IN SILICA AND ASBESTOS-INDUCED CARCINOGENESIS

Val Vallyathan, Min Ding, Xianglin Shi, Vincent Castranova

Pathology and Physiology Research Branch, Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Morgantown, West Virginia, USA

Occupational exposures to asbestos and crystalline silica have been implicated in causing lung cancer and other pulmonary diseases in humans. Despite intensive research during the last decade on pulmonary carcinogenesis induced by these minerals, the exact molecular mechanisms involved in carcinogenesis are still unknown. Chronic inflammation and enhanced production of reactive oxygen species (ROS) generated by these particulates have been implicated in the development of tumors. In an attempt to understand the molecular basis of carcinogenesis induced by these particles, we investigated the potential activation of activator protein-1 (AP-1) by crocidolite and freshly fractured or aged crystalline silica in a JB6 P^+ cell line stably transfected with AP-1-luciferase reporter plasmid (in vitro) and in AP-1-luciferase reporter transgenic mice (in vivo). This transcription factor governs the expression of target genes that are involved in encoding cytokines, chemokines, growth factors, cell adhesion molecules, and acute-phase proteins that regulate inflammation, cell proliferation, and apoptosis. Results of our studies suggest that asbestos and silica activate AP-1 through generation of ROS. In in vitro studies, crocidolite asbestos caused a dose- and time-dependent AP-1 activation in JB6+ cells, which persisted for at least 72 h. In transgenic mice exposed to crocidolite asbestos, AP-1 activation increased significantly by 10-fold in lung tissue and 22-fold in bronchial tissue. This induction of AP-1 activation by crocidolite appears to be mediated through the influence of mitogen-activated protein kinase (MAPK) family members, specifically extracellular signal-regulating protein kinase, ERK 1, and ERK 2 (data not presented). Similarly, freshly fractured silica caused an 8-fold increase in AP-1 activation in JB6 P+cells and 22-fold increase in transgenic mice. The activation of AP-1 by freshly fractured silica was mediated through ERK1, ERK2, and p38 kinase. Activation of AP-1 by asbestos or silica was inhibited in both in vitro and in vivo systems by aspirin, which exhibits OH radical scavenging properties. It is proposed from these studies that asbestos and crystalline silica may promote carcinogenesis through specific mechanistic pathways stimulated by ROS.

Occupational exposures to asbestos and crystalline silica have been implicated in causing lung cancer and other pulmonary diseases in humans (Mossman & Churg, 1998; Craighead et al., 1982). These minerals are also capable of inducing tumors in experimental animals and neoplastic transformation of cells in culture. Generally, these particles are negative in standard genotoxic assays and are thought to function differently than classical carcinogens. The exact mechanisms by which these inorganic particles induce carcinogenesis are still unknown. Chronic inflammation and enhanced production of reactive

Address correspondence to Val Vallyathan, PhD, NIOSH, 1095 Willowdale Road, Morgantown, WV 26505, USA. E-mail: vav1@cdc.gov

oxygen species (ROS) generated by these persistent particles have been implicated in the development of tumors (Driscoll et al., 1997; Vallyathan et al., 1998). Chronic inflammation may predispose the lung to carcinogenesis by the activation of growth mediators, cytokines, and oncogenic transformations (Vallyathan et al., 1998; Mossman et al., 1997). Therefore, identification of the specific molecular and biochemical pathways involved in cell transformation and tumor development in animals following exposure to these particles is important. In an attempt to understand the molecular basis of carcinogenesis induced by these particles, we investigated the potential activation of activator protein-1 (AP-1) by crocidolite and freshly fractured or aged crystalline silica in a JB6 P^+ cell line stably transfected with an AP-1-luciferase reporter plasmid (in vitro) and in AP-1-luciferase reporter transgenic mice (in vivo). We reported earlier that both crocidolite asbestos and crystalline silica induce AP-1 activation in cell culture system as well as in transgenic mice (Ding et al., 1999a, 1999b). Evidence presented in these studies has provided strong support for a significant role of ROS in promoting the activation of AP-1.

MATERIALS AND METHODS

The $JB6^+$ mouse epidermal cell line, a well-characterized system for studying tumor promotion and neoplastic transformation responses, was chosen as an in vitro model (Ding et al., 1999a, 1999b). JB6 P^+ cells were exposed to silica (200 μg/ml) or crocidolite (7.5 μg/ml) for 24 h or 36 h, respectively, at 37°C. At the termination of exposure, the cells were extracted with 200 μl of lysis buffer and luciferase activity was measured using a chemiluminometer. For in vivo studies, we used C57Bl/6 male mice, carrying the TRE-luciferase transgene, crossed with DBA2 females. These mice were intratracheally exposed to crocidolite asbestos, freshly fractured silica, aged silica, or vehicle (Ding et al., 1999a, 1999b). At 2 and 3 days after exposure, animals were sacrificed and lung and bronchiolar tissues were removed, homogenized, and lysed in lysis buffer overnight at 4°C. Luciferase activity was measured and expressed as relative AP-1 activity.

RESULTS

Exposure of mouse epidermal cells to crystalline silica or crocidolite resulted in activation of AP-1 (Figure 1). This activation was dose and time dependent (data not shown). For freshly fractured silica, significant activation was noted after a 24-h exposure to 80 μg/ml silica. As shown in Figure 1, freshly fractured silica was a more potent activator of AP-1 than aged silica. Significant activation of AP-1 was also noted after a 24-h exposure to 2.5 μg/ml crocidolite asbestos. This activation reached a maximum at 7.5 μg/ml crocidolite. At 7.5 μg/ml crocidolite, activation was significantly increased after 24 h of exposure, reached a maximum at 36 h, and was still elevated after 72 h (data not shown).

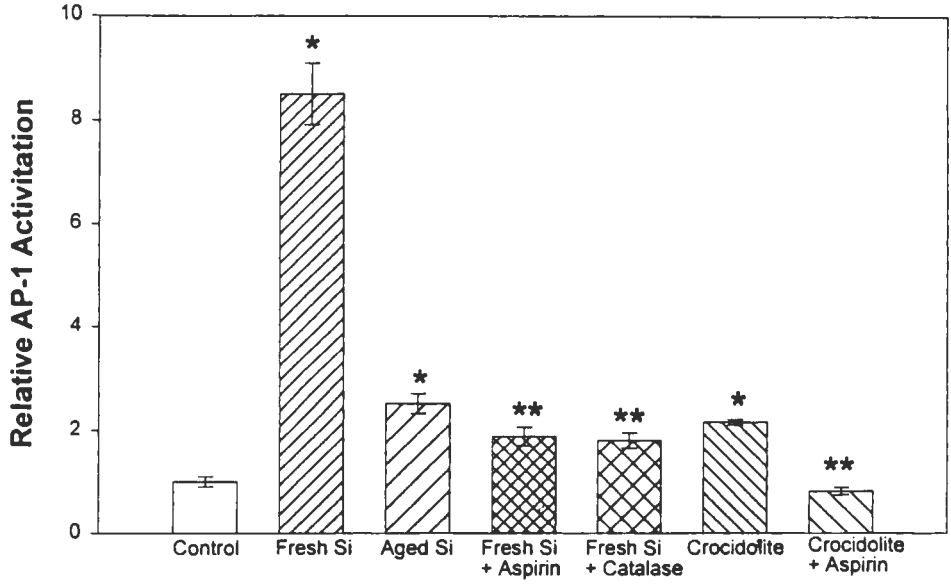

FIGURE 1. Silica- or crocidolite-induced AP-1 activation in JB6 P^+ cells. Mouse epidermal cells, transfected with AP-1-luciferase reporter plasmid, were exposed to silica (200 μg/ml) or crocidolite (7.5 μg/ml) for 24 h or 36 h, respectively. AP-1 induction was determined relative to control cells by measuring luciferase activity by chemiluminescence. Data presented are means and ± SE of at least eight determinations. Asterisk indicates a significant increase from control. Double asterisk indicates a significant decrease compared to fresh silica or crocidolite treated cells ($p \leq .05$).

ROS play a significant role in both silica- and crocidolite-induced AP-1 activation (Figure 1). AP-1 activation of epidermal cells in response to freshly fractured silica was inhibited by 90% in the presence of aspirin, a scavenger of RO5 (Shi et al., 1999). Aspirin also completely inhibited crocidolite-induced AP-1 activation in vitro. Freshly fractured silica and crocidolite also increased AP-1 activation of pulmonary tissue after intratracheal exposure of mice (Figure 2). AP-1 induction in response to crocidolite was increased 10-fold in lung tissue and 22-fold in bronchiolar tissue, while silica caused a 22-fold increase in AP-1 activation of whole lung tissue. As with the in vitro system, aspirin (120 mg/kg), administered intraperitoneally in transgenic mice 30 min prior to crocidolite instillation and every 12 h thereafter, significantly inhibited AP-1 activation by 89% (Figure 2).

DISCUSSION

These studies show that silica and asbestos activate AP-1 through the generation of RO5, which is a primary event important in the initiation of signal transduction cascades leading to the induction of early response genes that may be critical in carcinogenesis (Ding et al., 1999a, 1999b). Both asbestos and silica caused a time- and dose-dependent activation of AP-1 in cultured

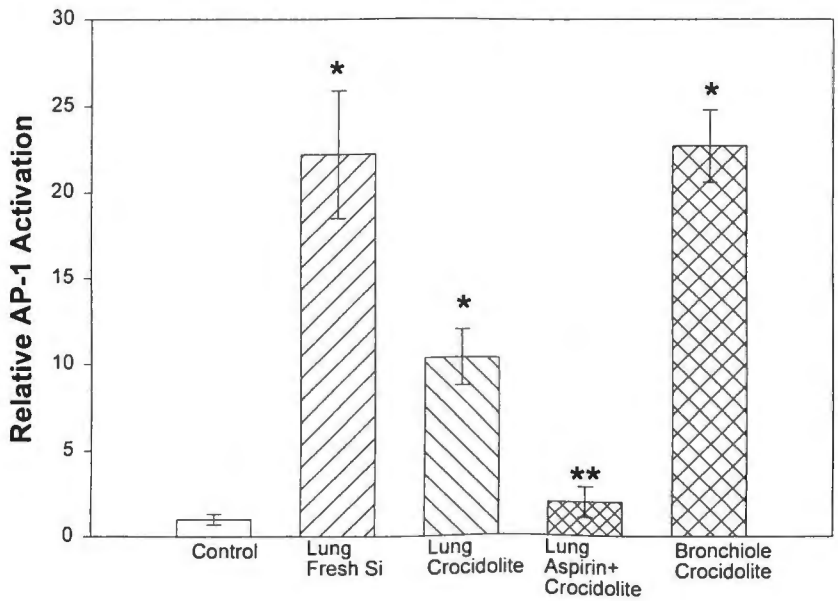

FIGURE 2. Silica- or crocidolite-induced AP-1 activation in mouse lung. AP-1-luciferase reporter transgenic mice were exposed by intratracheal instillation to 5 mg silica or 100 μg crocidolite and animals were sacrificed at 3 days post-exposure. AP-1 induction was determined relative to control (saline-treated) mice by measuring luciferase activity by chemiluminescence. Data presented are means and ± SE of at least eight animals. Asterisk indicates a significant increase from control. Double asterisk indicates a significant decrease from crocidolite with a scavenger ($p \leq .05$).

JB6 P^+ cells and transgenic mice (Ding et al., 1999a, 1999b). AP-1 activation reached a maximum at 200 μg/ml silica. With 200 μg/ml silica, activation was significantly increased after 12 h of exposure, reached a maximum at 24 h, and declined but was still elevated at 48–72 h. With crocidolite asbestos in mice, AP-1 activation was first observed at 2 days and reached a maximum after 3 days. Phosphorylation of extracellular signal-regulating protein kinase ERK1 and ERK2 appears to play an important role in silica- or crocidolite-induced AP-1 activation in epidermal cells (Ding et al., 1999a, 1999b). In addition, silica also stimulate increase phosphorylation of p38 protein kinase (Ding et al., 1999b). Aspirin, an RO5 scavenger, significantly inhibits silica or crocidolite-induced AP-1 activation. Therefore, we propose that the carcinogenic effect of crocidolite asbestos and crystalline silica may be mediated through the generation of $^{\cdot}OH$ leading to the induction of AP-1 activation. It is interesting to note that the effect of crystalline silica on AP-1 activation was an eightfold increase, compared to a moderate twofold increase with crocidolite (Figure 1). However, transactivation of AP-1 in transgenic mice was similar for lung in freshly fractured silica exposed and for bronchiolar tissue in crocidolite exposed animals. This may be related to the different pathways of activation of AP-1 and increased concentration of RO5 produced with silica used here at a higher

dose. These observations suggest that these minerals can promote molecular events linked to carcinogenesis independently of other confounding factors, such as tobacco smoke, frequently implicated in epidemiologic studies as a confounder.

REFERENCES

Craighead, J. E., Abraham, J. L., Churg, A., Green, F. H. Y., Kleinerman, J., Pratt, P. C., Seemayer, A., Vallyathan, V., and Weill, H. 1982. The pathology of asbestos associated diseases of the lungs and pleural cavities: Diagnostic criteria and proposed grading schema. *Arch. Pathol. Lab. Med.* 106:544–596.

Ding, M., Dong, Z., Chen, F., Pack, D., Ma, W. Y., Ye, J., Shi, X., Castranova, V., and Vallyathan, V. 1999a. Asbestos induces activator protein-1 (AP-1) transactivation in transgenic mice. *Cancer Res.* 59:1884–1889.

Ding, M., Shi, X., Dong, Z., Chen, F., Lu, Y. J., Castranova, V., and Vallyathan, V. 1999b. Freshly fractured crystalline silica induces activator protein-1 activation through ERKs and p38 MAPK. *J. Biol. Chem.* 274:30611–30616.

Driscoll, K. E., Deyo, L. C., Carter, J. M., Howard, B. W., Hassenbein, D. G., and Bertram, T. A. 1997. Effects of particle exposure and particle-elicited inflammatory cells on mutation in rat alveolar epithelial cells. *Carcinogenesis* 18:423–430.

Mossman, B. T., and Churg, A. 1998. Mechanisms in the pathogenesis of asbestosis and silicosis. *Am. J. Respir. Crit. Care Med.* 157:1666–1680.

Mossman, B. T., Faux, S., Janssen, Y., Jimenez, L. A., Timblin, C., Zanella, C., Goldberg, J., Walsh, E., Barchowsky, A., and Driscoll, K. 1997. Cell signaling pathways elicited by asbestos. *Environ. Health Perspect.* 105:1121–1125.

Shi, X., Ding, M., Dong, Z., Chen, F., Ye, J., Wang, S., Leonard, S. S., Castranova, V., and Vallyathan, V. 1999. Antioxidant properties of aspirin: Characterization of the ability of aspirin to inhibit silica-induced lipid peroxidation, DNA damage, NF-κB activation and TNF-α production. *Mol. Cell. Biochem.* 199:93–102.

Vallyathan, V., Shi, X., and Castranova, V. 1998. Reactive oxygen species: Their relation to pneumoconiosis and carcinogenesis. *Environ. Health Perspect.* 106:1151–1155.