Expression of Matrix Metalloproteinases, Tissue Inhibitors of Metalloproteinases, and Extracellular Matrix mRNA Following Exposure to Mineral Fibers and Cigarette Smoke in Vivo

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To determine the effect of mineral fibers and cigarette smoke on remodeling of lung tissues, we examined matrix metalloproteinase-1 (MMP-1), MMP-2, tissue inhibitors of metalloproteinase-1 (TIMP-1), TIMP-2, and types I and IV collagen mRNA levels from rat lungs exposed to mineral fibers and/or cigarette smoke in vivo. Male Wistar rats (10 weeks of age) were given a single intratracheal instillation of 2 mg of chrysotile or alumina silicate ceramic fibers (RCF). Animals were then exposed to cigarette smoke (side stream) 5 days per week for 4 weeks. Transcriptional levels of mRNA extracted from the lungs were assessed by semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR). Exposure to cigarette smoke induced increases in MMP-1 and TIMP-1 mRNA levels and decreased TIMP-2 and type I collagen mRNA levels in lung. Chrysotile or RCF stimulated the expression of MMP-1 mRNA in the lung. The mineral fibers and cigarette smoke had more additive effects on the expression of MMP-2 and TIMP-1 in the lung. These data suggest that the imbalance of the expression of MMPs, TIMPs, and extracellular matrix may be associated with the remodeling of lung tissues induced by mineral fibers and/or cigarette smoke. — Environ Health Perspect 105(Suppl 5):1247-1251 (1997)

Key words: MMP, TIMP, asbestos, lung, ceramic fiber, chrysotile, collagen

Introduction

Ocupational and environmental inhalation of asbestos and various types of man-made mineral fibers is thought to cause remodeling of lung tissues that can result in fibrosis (1-2). Furthermore, some reports show that combined exposure to mineral fibers and cigarette smoke may result in remodeling of lung tissues (3).

Extracellular matrix (ECM) degradation presumably contributes to the initial phase of tissue remodeling inherent to the physiological processes of morphogenesis, angiogenesis, inflammation, and wound healing (4). Matrix metalloproteinases (MMPs) are a family of zinc and calcium-dependent endopeptidases that play a key role in ECM remodeling in the lung (5). MMPs have the combined ability to degrade the various components of connective tissue matrices specifically directed to ECM components (4,5). However, the activities of MMPs are controlled at several levels including their interactions with specific inhibitors, e.g., the tissue inhibitors of metalloproteinases (TIMPs) (5). MMP-1 is an interstitial collagenase that specifically degrades connective tissue fibrillar collagen (type I collagen), and MMP-2 is a gelatinase that degrades type IV globular basement membrane collagen (6). The representative specific inhibitors MMP-1 and MMP-2 are TIMP-1 and TIMP-2, respectively (5). An imbalance of MMPs and TIMPs results in metalloproteinase activation, and relatively higher levels of MMPs than TIMPs may stimulate the degradation of collagen in the interstitial space and beneath epithelial and endothelial cells, leading to acute injury (6).

To determine the effect of the mineral fibers and cigarette smoke on remodeling of rat lung tissues, we examined MMP-1, MMP-2, TIMP-1, TIMP-2, and types I and IV collagen mRNA levels from rat lungs exposed to mineral fibers and cigarette smoke in vivo, using reverse transcription-polymerase chain reaction (RT-PCR).

Materials and Methods

Fibers

The fibers used in this study were chrysotile (Union Internationale Contre le Cancer) and alumina silicate ceramic fibers (RCF) (Japan). For chrysotile the geometric mean diameter (SD), geometric mean length, and number/mass were 0.085 (1.4) μm, 0.7 (1.9) μm and 4.0×10³/lug, respectively. For RCF the SD and geometric mean length were 1.2 (1.7) μm and 9.6 (1.9) μm, respectively. In our previous report, the detailed physical and chemical characteristics of these fibers were investigated (7).

Experimental Design

Male Wistar Rats (10 weeks of age) were used in this study, and thirty rats were divided into six groups (saline, chrysotile, RCF, cigarette smoke, chrysotile+ cigarette smoke, RCF+cigarette smoke). Saline or chrysotile suspension or ceramic suspension (2 mg/0.2 ml) was administered to rats intratracheally. Rats were housed in an exposure chamber (8), and 20 cigarettes were smoked for 4 hr/day, 5 days/week for 4 weeks. The concentration of cigarette smoke in the chamber was 10 mg/m³, as measured gravimetrically from glass fiber samples. The chamber volume was 100 liters and the flow rate in the chamber was 50 liters/min.

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This paper is based on a presentation at The Sixth International Meeting on the Toxicology of Natural and Man-Made Fibrous and Non-Fibrous Particles held 15-18 September 1996 in Lake Placid, New York. Manuscript received at EHP 26 March 1997; accepted 2 April 1997.

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Abbreviations used: ECM, extracellular matrix; MMP, matrix metalloproteinase; NIH, National Institutes of Health; NS, normal saline; PCR, polymerase chain reaction; RCF, refractory ceramic fiber; RT-PCR, reverse transcription-polymerase chain reaction; SD, geometric mean diameter; TIMP, tissue inhibitor of metalloproteinase.
Preparation of RNA, cDNA Synthesis, and Polymerase Chain Reaction

RNA was extracted from the lung using guanidinium thiocyanate–phenol–chloroform (9). Total RNA (0.5 µg) was used for the synthesis of single-strand cDNA using Moloney murine leukemia virus-derived reverse transcriptase as described by Wang and Mark (10). The amplification was performed with a Thermocycler (Astech, Japan) under the following conditions: 94°C for denaturation for 45 sec, 60°C for annealing for 45 sec, and 72°C for extension for 2 min for MMPs, TIMPs, collagen, and β-actin genes. β-actin was coamplified as an internal standard to quantitative polymerase chain reaction (PCR) amplification of mRNA (10). PCR products were resolved by gel electrophoresis and visualized by ethidium bromide staining. The gels were photographed with Polaroid Type 665 positive/negative film (Polaroid Corporation, Cambridge, MA) over UV light at the same exposure and developing time. The bands of the positive film were scanned and the density of each PCR product was measured using National Institutes of Health (NIH) image 1.55 software (written by W. Rasband at NIH, Bethesda, MD). To quantify the transcriptional level of mRNA, the data were normalized to represent equivalent RNA loading based on the density of β-actin at the appropriate cycle of a given gene product (10).

Histopathology

Lungs were fixed in 10% buffered formalin. Embedded samples were then sectioned and stained with hematoxylin and eosin for microscopic evaluation.

Results

Expression of MMP-1 and MMP-2 mRNA in Lungs

The results of changes in the abundance of MMP-1 mRNA in the lung are shown in Figure 1. In comparison with the saline control group, levels of MMP-1 mRNA in lungs were increased significantly in animals treated with chrysotile, RCF, and/or cigarette smoke. Lung MMP-1 mRNA in the group exposed to chrysotile+cigarette smoke was maximally expressed relative to all groups. Elevations in MMP-2 mRNA were not observed in lungs exposed to mineral fibers or cigarette smoke. However, the level of MMP-2 mRNA was elevated significantly in rat lungs exposed to chrysotile+cigarette smoke and RCF+cigarette smoke (Figure 2).

Expression of TIMP-1 and TIMP-2 mRNA in Lungs

Transcripts for TIMP-1 in the control- or mineral fiber-treated lung were not detected (Figure 3). However, gene expression of TIMP-1 in groups exposed to cigarette smoke and/or mineral fibers was observed. Combined effects of chrysotile and cigarette smoke on message level of TIMP-1 in rat lungs were observed.

Although mineral fibers did not change TIMP-2 mRNA levels in the lung, cigarette smoke markedly decreased levels of TIMP-2 mRNA in the lung. Combined effects of the two agents were not observed (Figure 4).

Expression of Types I and IV Collagen mRNA in Lungs

In rats exposed to mineral fibers, mRNA levels for type I collagen in the lung were not increased. Cigarette smoke decreased in type I collagen mRNA levels in rat lungs (Figure 5). Message for type IV collagen in the lung was not detected in any of the groups except in a positive control, although the cycle number for PCR in these experiments was up to 40.

Pathological Findings

Foreign body giant cells and mononuclear cells around bronchioles were observed in
groups exposed to chrysotile and chrysotile + cigarette smoke (Figure 6). In the other four groups, definite pathological changes were not observed.

Discussion

MMP-1, TIMP-1, and Type 1 Collagen

Mineral Fibers and Cigarette Smoke. RT–PCR revealed that exposure to mineral fibers, especially chrysotile, induced increases in MMP-1 mRNA levels in rat lungs. A previous report (11) showed that exposure to 85% O₂ leads to an early increase in gene expression of the interstitial collagenase MMP-1 in acute lung injury. Our results were consistent with such an injurious response. In our experiment, cigarette smoke elevated levels of MMP-1 mRNA in the lung. Cigarette smoking is the overwhelming factor associated with pulmonary emphysema (12). Heckman and Dalbey (13) reported that cigarette smoke alone can produce emphysema in rats exposed for long periods of time. D’Armiento et al. (14) reported that MMP-1 overexpression in the lungs of transgenic mice causes pulmonary emphysema. Therefore, the upregulation of MMP-1 by cigarette smoke might be related to the destruction of ECM.

It was reported that the ratio of type I to type III collagen is greatly increased in individuals dying of idiopathic pulmonary fibrosis (15). However, exposure to mineral fibers did not lead to an increase in gene expression of type I collagen in rat lungs. Arden and Adamson (16) found that intratracheal injection of crocidolite in rats caused an increase in the ratio of type III collagen to type I collagen at the first 4 weeks, followed by a shift to a higher proportion of type I collagen. The time course of gene expression of collagen in lungs following initial asbestos exposure is now under investigation.

Cigarette smoke markedly decreased the levels of type I collagen mRNA in lungs. Cigarette smoke may not only stimulate the degradation of collagen, but also may suppress the production of collagen, leading to emphysema.

Combined Exposure to Mineral Fibers and Cigarette Smoke. Chrysotile and cigarette smoke did not have a combined effect on MMP-1 mRNA in the lung. However, in two groups (chrysotile and chrysotile + cigarette smoke) showing high expression of MMP-1 in the lung, the pathological features of inflammation were observed. MMP-1 in the lung may facilitate inflammatory cell migration and induce ECM solubilization, subsequently extending the area of connective tissue destruction (6). In this sense, MMP-1 in the lung may contribute to the remodeling of lung tissues exposed to chrysotile and/or cigarette smoke. An additive, combined effect of two agents on the gene expression of TIMP-1 in the lung was observed in our study. This phenomenon might represent a compensatory mechanism against increases in MMP-1; it has been reported that TIMP suppressed the aberrant activation of MMP (17), and TIMPs and MMPs are coexpressed in many cell types in response to a variety of inducing stimuli (18).

MMP-2, TIMP-2, and Type IV Collagen

Mineral Fibers and Cigarette Smoke. In our study, single exposure to mineral fibers or cigarette smoke alone did not cause an increase in levels of MMP-2 mRNA in the lung. D’Ortho et al. (19) demonstrated an increase in the gelatinase and stromelysin activities in the bronchoalveolar lavage fluid in the course of acute lung injury in rats exposed to ozone. However, MMP-2, unlike other MMPs, was not affected by proinflammatory cytokines or PMA, which suggests a specific regulation of MMP-2 (20).

The gene of TIMP-2 was expressed in the saline-exposed lung, whereas exposure to mineral fibers did not induce message levels of TIMP-2. TIMP-2 is expressed in many normal tissues; mRNA is expressed in most mesenchymal-derived tissues (21,22). A series of ras-transformed derivatives of C3H10T1/2 fibroblasts showed great variability in TIMP-1 expression, whereas TIMP-2 expression was insensitive to transformation (22). However, we found that TIMP-2 mRNA levels in the lung were markedly decreased by cigarette smoke, which might result in the elevation of gelatinolytic activities. Skup and Ponton (23) reported that increased metalloproteinase activity in different pathologies could be due to decreased levels of the specific TIMP. In our experiments, the message for type IV collagen in the lung was not detected in all groups. The primer for type IV collagen was based on the sequence of the mouse. Using the same primer, Kaneto et al. (24) reported that the level of type IV collagen mRNA measured by RT–PCR was increased in rat kidney with interstitial fibrosis but undetectable in control kidney. Hence, our failure to detect type IV collagen mRNA was not related to primer selection.
Combined Exposure to Mineral Fibers and Cigarette Smoke. Combined exposure to mineral fibers and cigarette smoke markedly induced an increase in levels of MMP-2 mRNA in the lungs. Therefore, increases in MMP-2 induced by the two agents suggest a possible elevation of gelatinolytic activity that could degrade basement membrane. Nery et al. (25) reported additive effects of smoking and exposure to silica dust in increasing epithelial permeability in patients with silicosis who smoke.

Conclusion

In summary, we examined gene expression of MMPs, TIMPs, and collagen in the rat lungs exposed to mineral fibers and/or cigarette smoke. Mineral fibers and cigarette smoke had combined effects on the expression of MMP-2 and TIMP-1 in the lungs. These data suggest that MMPs and TIMPs may be associated with remodeling of lung tissues induced by mineral fibers and cigarette smoke.
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