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INCREASES IN ENDOGENOUS ANTIOXIDANT ENZYMES DURING ASBESTOS INHALATION IN RATS

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Although the pathogenesis of asbestos-induced pulmonary damage is still not completely understood, an important role has been attributed to active oxygen species. In the present paper we present results of a study investigating the effect of crocidolite asbestos inhalation on different lung antioxidant enzymes in rats. During the development of pulmonary fibrosis induced by crocidolite asbestos, lung superoxide dismutase, catalase and selenium-dependent glutathione peroxidase activities increased, indicating an adaptive response to increased pulmonary oxidant stress. However, this adaptive response obviously is not sufficient to protect the lung from asbestos-induced pulmonary damage. Considering the role of active oxygen species in both the fibrotic process and tumor promotion, it is hypothesized that antioxidants may also protect the lung from chronic asbestos-induced pulmonary damage such as bronchogenic carcinoma.

KEY WORDS: Antioxidants, asbestos, fibrosis.

INTRODUCTION

Occupational exposure to asbestos is associated with an increased risk of asbestosis, bronchogenic carcinoma and mesothelioma.[1] Several studies indicate that active oxygen species are important mediators in the pathogenesis of asbestos- induced lung pathologies. Active oxygen species are released by inflammatory cells after phagocytosis of asbestos fibers.[2] Moreover, it has been demonstrated that active oxygen species are generated from asbestos fibers in a cell free system containing H_2O_2,[3] xanthine oxidase or NADH diaphorase in the presence of an auto-oxidizable quinone,[4,5] or in an aqueous environment.[6]

In *in vitro* studies asbestos-induced cytotoxicity is inhibited by addition of antioxidants[7-10] indicating a probable role of oxygen radicals in cell damage. Lipid peroxidation can be prevented in peritoneal macrophages exposed to asbestos *in vitro* by antioxidant administration as well.[11]

Furthermore, oxygen radicals may also play a role in asbestos-induced pulmonary

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fibrosis. Active oxygen species can cause direct pulmonary injury[12] and subsequent compensatory hyperplasia might disturb the normal regulation of collagen metabolism, leading to pulmonary fibrosis.[7] In support of this hypothesis, it has been shown that production of superoxide anion generated by xanthine-xanthine oxidase increases both collagen and non-collagen protein metabolism in rat lung fibroblasts as does crocidolite asbestos.[2]

The exact mechanisms of asbestos-induced malignancy remain unclear. Different cell types of the lung respond differently to asbestos and this might be linked to the predilection of certain cells (e.g. mesothelial cells) towards transformation.[13] Asbestos is a complete carcinogen in mesothelial cells, the progenitor of mesothelioma. In contrast, the role of asbestos in the development of bronchogenic carcinoma appears to be primarily that of a tumor promoter,[13] for when added to tracheal epithelial cells, asbestos causes morphological and biochemical alterations indentical to those seen with application of classic tumor promoters. These include induction of cell division, changes in normal cell differentiation, induction of ornithine decarboxylase (ODC) activity,[14,15] activation of phospholipase[16] and activation and translocation of protein kinase C.[17] These studies corroborate accumulating evidence from several investigators suggesting that the mode of action of tumor promoters might be through oxidant mechanisms.[18]

Asbestosis is one of many pulmonary disorders such as respiratory distress syndrome and ozone- or tobacco smoke-induced pulmonary damage which is associated with increased oxidant stress. Cells contain a battery of compounds including antioxidant enzymes such as catalase, superoxide dismutase (SOD) and glutathione peroxidase (GPX) which protect against the toxic effects of oxidants. *In vivo* studies have shown that pulmonary antioxidant enzymes are increased in rat lung after sublethal exposure to hyperoxia or endotoxin (see review 19). In related studies, we have shown that the diffuse pulmonary fibrosis observed in rats after a 2–4 week inhalation of crocidolite could be blocked 'pharmacologically' with catalase administration.[20] In the present study, we examined antioxidant enzymes in rat lung after a 2 week exposure to crocidolite asbestos to see if asbestos inhalation induces an adaptive pulmonary response in rats as do other types of oxidant stress.

METHODS

Exposure

Male Fischer 344 rats, weighing approximately 200–250 grams, were exposed to N.I.E.H.S. crocidolite asbestos (10 mg/m^3) for 6 hours/day, 5 days/week for 10 days. Asbestos fibers were generated using a modified Timbrell dust generator.[21] Sham control animals were placed in dust free chambers and handled identically.

Chemicals

Catalase was purchased from Worthington Biochemical Co., St. Louis, MO, SOD from Data Diagnostics Inc., Mountain View, CA and Biorad dye reagent from Biorad, Richmond, CA. All other chemicals and reagents were obtained from Sigma Chemical Co., St. Louis, MO.

Determination of Endogenous Antioxidant Enzymes

After 1,3,6 and 9 days of exposure and at 14 days after cessation of exposure rats were sacrificed with pentobarbital, the chest was opened and the lungs were perfused with heparinized Ca^{2+} Mg^{2+} free phosphate buffered saline (CMFPBS) via the vasculature until the lungs appeared white. Lungs were removed, rinsed, trimmed and homogenized at 4°C (Polytron, Brinkman, Westbury, NY). Samples were centrifuged at 14,000 rpm for 10 minutes at 4°C and supernatants stored at −70°C until antioxidant enzyme analyses. Catalase was determined as described by Beers and Sizer[22] measuring decomposition of H_2O_2 at 240 nm. Selenium-dependent glutathione peroxidase (GPX) was determined measuring oxidation of NADPH at 340 nm and using H_2O_2 as substrate.[23] Total superoxide dismutase (SOD) was measured by determining the amount of sample required to produce 50% inhibition of the cytochrome *c* reduction generated by a stable xanthine-xanthine oxidase reaction,[24] (50% inhibition = 1 unit SOD). In a control experiment the enzymatic nature of the inhibition of cytochrome *c* reduction in the lung was checked by heat inactivation and cyanide inhibition. No inhibitory activity was found after boiling of the sample at 100°C for five minutes whereas more than 90% inhibition of the total activity was observed after incubation of the sample with 10 mM sodium cyanide (data not shown) indicating that inhibition of cytochrome *c* reduction is due to SOD activity in the lung tissue. The protein content of the lung tissue was measured according to the method of Bradford.[25]

Statistics

All results were evaluated using the "SPSX-program" (SPSS-X inc.). Before testing histograms were produced from all parameters in order to determine their frequency

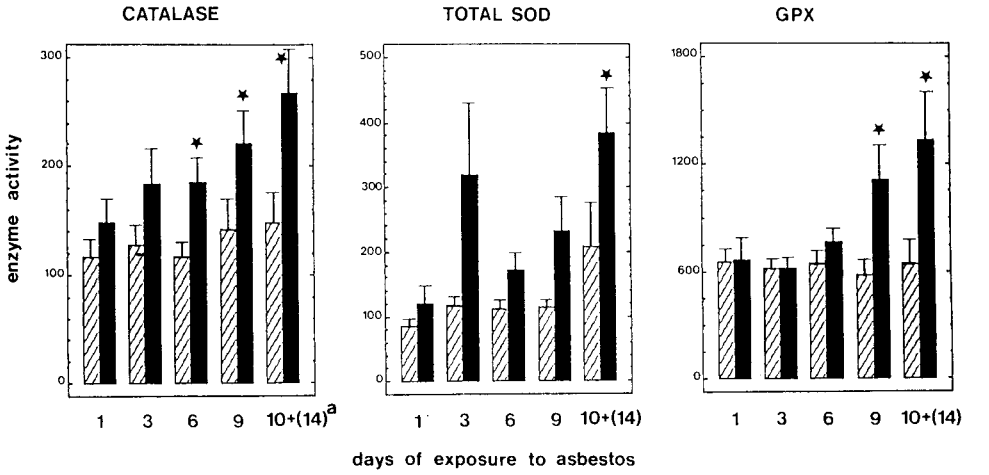

FIGURE 1 Lung antioxidant enzyme activities after 1,3,6 and 9 days of exposure to crocidolite asbestos and at 14 days after cessation of exposure. Enzyme activities are expressed in U/mg protein (catalase and SOD) or mU/mg protein (GPX). Data are mean + standard error of 3 experiments (n = 11–12); *significantly different (Mann Whitney, $P < 0.05$) compared to sham controls. There was a significant linear trend over time for catalase and GPX (Kruskal-Wallis, $P < 0.05$) in crocidolite exposed rats. Shaded bars are controls and black bars crocidolite asbestos-exposed rats. [a]10 days of exposure plus 14 days of maintenance in clean air.

distributions. The Mann Whitney U test[26] was used in order to detect differences between control and crocidolite exposed groups on each exposure day and a trend analysis using the Kruskal-Wallis test determined if there was a significant increase in enzyme activity with time.

RESULTS

Figure 1 shows antioxidant enzyme activities in lung homogenates during the exposure to crocidolite asbestos. Catalase activity increased significantly after 6 and 9 days of exposure to crocidolite and 2 weeks after cessation of exposure. Lung GPX activity was increased significantly after 9 days of exposure and at 14 days after cessation of exposure, whereas total SOD was increased significantly only at the last time point, i.e. 14 days after cessation of exposure. The present exposure protocol, although shorter than reported previously[20] still resulted in pulmonary fibrosis which was confirmed by histopathology and hydroxyproline content (data not shown).

DISCUSSION

The present data show that the activities of pulmonary antioxidant enzymes increase significantly during the development of asbestosis in rats. These findings are indicative of an adaptive response to increased oxidant stress imposed by asbestos fibers. This physiological response is not sufficient to confer protection against pulmonary damage, however, for a number of possible reasons. One explanation might be the consequence of a phenomenon unique to particulate exposure called "frustrated phagocytosis" where the phagolysosome of macrophages cannot close completely during phagocytosis of long fibers. This results in a continuous and prolonged release of oxygen radicals and other inflammatory intermediates which might overwhelm the natural autooxidant defenses of the lung. In addition, our past studies show that a high dosage of 'therapeutically' administered exogenous catalase is required to boost rat serum levels to the extent (10-fold higher than control levels)[27] that significant protection from asbestosis occurs during simultaneous exposure to asbestos.[20] Delivery of the enzyme was undoubtedly enhanced by conjugation to polyethylene glycol (PEG) which can promote cellular uptake,[28] reduce antigenticity[29] and facilitate an increase in enzyme content of the rat lung after systemic administration.[27] Nonetheless, it appears unlikely that the twofold induction of endogenous antioxidant enzymes achieved in this study would be sufficient to protect rat lungs from fibrosis in view of the amount of exogenous catalase required to achieve protection.

A second possible reason for the lack of protection is that the extent of antioxidant enzyme induction would be sufficient to protect against large fluxes of oxygen radicals if induction were rapid, but the response occurs slowly and after irreversible asbestos damage has occurred and is therefore not protective. As shown in Figure 1, lung catalase activity does not increase significantly until after 6 days of exposure to crocidolite while lung SOD and GPX activities do not increase until even later time points.

Lastly, the possibility exists that antioxidant enzymes are not increased in all areas of the lung, that is, increases may be localized to certain cells or cell compartments leaving others unprotected.

Although our studies with PEG-catalase indicate that antioxidants, present in large

amount, available to the total lung tissue and present from the beginning of exposure, can protect the lung effectively from acute pulmonary damage and fibrosis induced by asbestos it is not known whether antioxidant administration would also protect the lung from asbestos-induced malignancy that might develop at the end of the normal life span of a rat. If the early pulmonary damage induced by asbestos is critical to both the elaboration of pulmonary fibrosis and subsequent development of cancer, the current findings suggest that exogenous adminstration of antioxidants would also protect against asbestos-induced malignancy. In support of this, others have hypothesized that the development of pulmonary fibrosis is a prerequisite for the development of asbestos-induced malignancy.[30,31]

In summary, we have shown that short term crocidolite inhalation in rats results in a 2-fold elevation in antioxidant enzymes (catalase, SOD, GPX). However, these increases are not sufficient to protect the lung from oxidant damage. It is hypothesized that antioxidants in appropriate conditions should protect the lung form chronic asbestos-induced bronchogenic carcinoma.

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