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APPROACHES TO PREVENTION OF ASBESTOS-INDUCED LUNG DISEASE USING POLYETHYLENE GLYCOL (PEG)-CONJUGATED CATALASE

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Abstract—Asbestos-associated damage to cells of the respiratory tract in vitro can be prevented by the simultaneous addition of scavengers of active oxygen species to cultures. To determine if administration of scavenger enzymes to animals and humans is a plausible approach to the prevention of asbestos-induced lung disease, osmotic pumps were filled with various concentrations of PEG-coupled catalase and implanted subcutaneously into Fischer 344 rats over a 28-day period. At 3, 14, and 28 days after implantation of the pumps, the animals were evaluated for levels of catalase in serum and lung. In addition, lung tissue and lavage fluids were examined at 28 days for biochemical and morphologic indications of cell injury, inflammation, and fibrotic lung disease. At all time points examined, the administration of PEG-catalase caused a dosage-dependent increase in serum levels of catalase. The levels of lung catalase were evaluated at 28 days but not at earlier time periods. In comparison to control rats, the amounts of enzymes (lactic dehydrogenase, alkaline phosphatase), protein, and cells in lavage fluids from treated animals were unaltered. Moreover, the lungs showed no evidence of inflammation or fibrotic disease as determined by differential cell counts in lavage and measurement of hydroxyproline. These studies suggest that administration of PEG-catalase does not cause injury or other alterations in lung tissue and can be pursued as a feasible approach to prevention of asbestosis.

Keywords—Asbestos, Asbestosis, Lung injury, Oxygen free radicals, Lactic dehydrogenase, Alkaline phosphatase, PEG, Catalase

INTRODUCTION

Asbestos is a family of minerals causally associated with the development of malignant (bronchogenic carcinoma, mesothelioma) and fibrotic (asbestosis) lung disease in man.[1] In an effort to develop preventive approaches to disease, work in this laboratory has focused on elucidating the mechanisms of asbestos-induced injury to cells of the respiratory tract. Since asbestos perturbs the plasma membrane before its uptake by cells[2] and contains metals (e.g., iron) on the surface of fibers that could drive reactions favoring the production of active oxygen species, we speculated that the mineral might cause the generation of oxygen free radicals from cells of the respiratory tract. Subsequently, active metabolites of oxygen might trigger cytotoxic and proliferative alterations intrinsic to the pathogenesis of disease. In support of our theory, we were able to prevent asbestos-associated damage to tracheobronchial epithelial cells, fibroblasts, and alveolar macrophages in vitro after the addition of superoxide dismutase (SOD), catalase, and scavengers of the hydroxyl radical (OH·) (mannitol, sodium benzoate, dimethylthiourea) to cultures.[3–5]

A goal of work presently in progress is the amelioration of fiber-induced disease in a rapid-onset inhalation model of asbestosis.[6] In brief, Fischer 344 rats exposed to crocidolite asbestos (10 mg/m^3 air) for a 4-week period (5 days/week, 6 h/day) develop focal fibrotic lesions and elevated amounts of hydroxypro-

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line in the lung. These animals also exhibit inflammatory changes in the lung as documented by histology and increased numbers of polymorphonuclear leukocytes (PMNs) and lymphocytes in differential cell counts on lavage fluids. In comparison to sham controls, protein, lactic dehydrogenase (LDH), and alkaline phosphatase in lavages from asbestos-exposed rats are also elevated significantly. The objectives of the work described below were to (1) develop a practical means for long-term administration of scavenger enzymes to rats exposed to asbestos, (2) determine whether this approach boosted the amounts of scavenger enzymes in the serum and lung of treated animals, and (3) establish that normal lung is not perturbed after administration of scavenger enzymes. In experiments here, we focused on the use of catalase conjugated to polyethylene glycol (PEG) in an effort to increase the circulating half-life of the enzyme.[7] Conjugation of catalase to PEG also masks possible antigenic sites and promotes its fusion with and entrance into cells of the respiratory tract.[8]

MATERIALS AND METHODS

Implantation of osmotic pumps into rats

PEG-catalase (specific activity = 17,290 IU/mg protein) was obtained from ENZON, Inc. (South Plainfield, NJ), diluted in Earle's balanced salt solution (GIBCO Laboratories, Grand Island, NY), and loaded by syringe into Alzet 28-day osmotic pumps with a 2.1-ml capacity (Alza Corporation, Palo Alto, CA). Pumps containing enzyme were implanted subcutaneously into 200-g male Fischer 344 rats (Charles River, Kingston, NY) divided into three groups (N = 4/group/time period), receiving 800, 4,000, and 8,000 IU PEG-catalase per day, respectively. Sham animals were anesthetized using sodium pentobarbital, and an incision was made and closed with autoclips. However, no pumps were implanted in these rats.

Measurement of catalase in serum and lung

At 3, 14, and 28 days, sham and catalase-treated rats (N = 4/group) were anesthetized with sodium pentobarbital and the renal artery was clipped to allow collection of whole blood. The lungs of additional animals were perfused through the vasculature, with phosphate-buffered saline (PBS, pH 7.4), excised, minced, and homogenized in 0.05 M of phosphate butter (pH 7.8) containing 0.1% Triton X 100, using a Polytron apparatus (Brinkmann, Westbury, NY), and centrifuged at 10,000 × g for 10 min. Lung supernatants and sera were then assayed for catalase using the technique described by Beers and Sizer.[9]

Assays for determination of lung injury, inflammation, and fibrosis

At 28 days, the animals were lavaged *in situ* using a closed chest technique. After cannulation of the trachea, a total volume of 35 ml Ca^{++}- and Mg^{++}-free PBS was infused into the lung using 5-ml aliquots. Lavage fluid was collected in a centrifuge tube by gently massaging the lungs after each instillation. After centrifugation at 2000 × g, supernatants were used for determination of LDH,[10] alkaline phosphatase,[11] and protein.[12] Cell pellets from lavage were resuspended in 20 ml Minimal Essential Medium (GIBCO) containing 10% fetal calf serum and counted using a hemocytometer. Cytospin smears from each preparation were prepared using a cytocentrifuge (Shandon, Swickeley, PA), fixed in methanol, and stained with May-Grunwald Giemsa. Differential cell counts to determine the percentage of pulmonary alveolar macrophages, PMNs, and lymphocytes were obtained by counting at least 1,000 cells per slide on a light microscope (400 magnification). The lungs were perfused as described above, excised, and the right upper lobe placed in a glass petri dish and dried at 60°C overnight. Total collagen content was then determined by measuring hydroxyproline by the procedure of Woessner.[13]

RESULTS

One goal of the experiments here was to elevate catalase levels for prolonged periods in the sera and lungs of the treated animals. Figure 1 shows the amounts of enzyme detected over a 28-day period after administration of PEG-catalase to rats in osmotic pumps. In comparison to the sham rats, the animals treated with PEG-catalase showed a dosage-dependent increase in the level of enzyme at 3, 14, and 28 days. The level of catalase in lung was not altered significantly at 3 and 14 days. However, a significant, dosage-dependent increase in enzyme was observed in lungs at 28 days.

Amounts of LDH, alkaline phosphatase, and protein in lavage fluids were measured to determine if lung injury occurred after administration of PEG-catalase. As can be seen in Figure 2, the enzyme and protein levels in lavages from the treated animals were not elevated significantly above the levels observed in controls. For reasons that are unclear, hydroxyproline, an indication of collagen content, was decreased in rats receiving 800 U/day PEG-catalase. However, no changes in the amount of the amino acid in lung were seen at higher concentrations of PEG-catalase.

The total number of cells in the lavage fluids of rats treated with PEG-catalase was unaltered in comparison

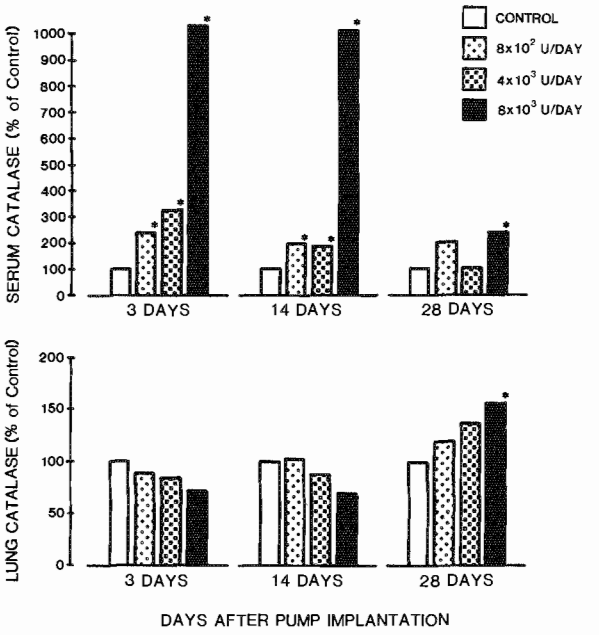

Fig 1. Levels of serum and lung catalase in rats receiving PEG-catalase in osmotic pumps. Values are expressed as a percentage of levels found in sham animals $*P < 05$ with respect to sham animals using Student's *t*-test.

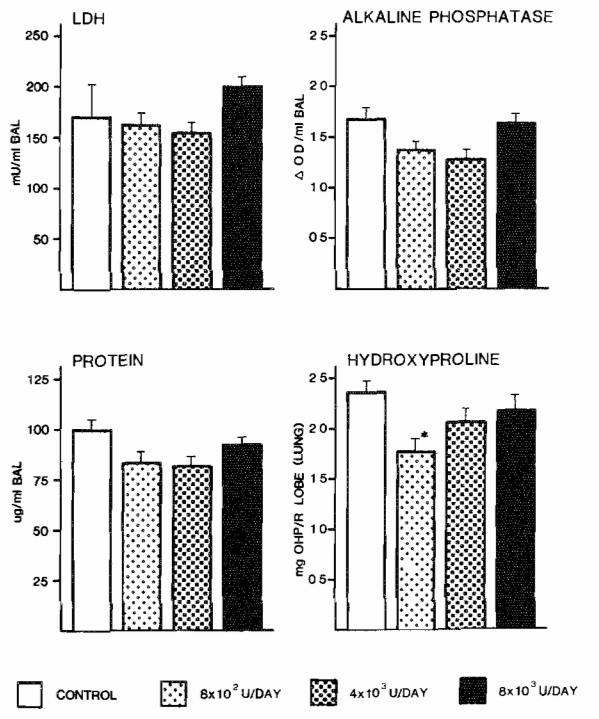

Fig 2. Amounts of LDH, alkaline phosphatase, and protein in lavage fluids at 28 days in sham controls and rats receiving PEG-catalase. Hydroxyproline was also measured as an indication of collagen content of lung. $*P < .05$ with respect to sham animals using Student's *t* test.

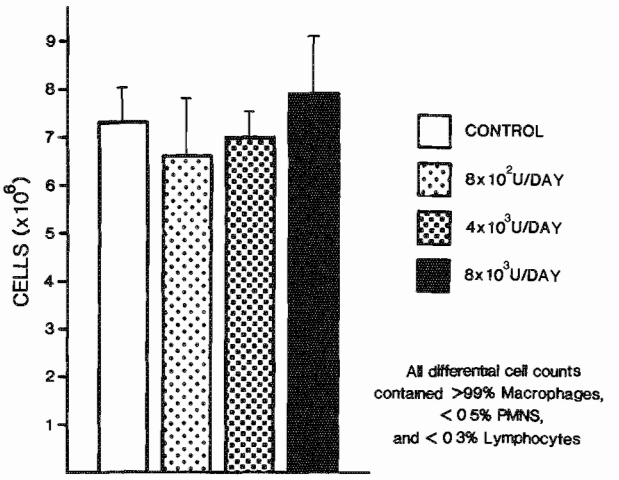

Fig 3 Total cell counts in lavages from sham and PEG-catalase treated rats at 28 days after implantation of osmotic pumps.

to sham controls (Fig. 3). In all groups, differential cell counts indicated that over 99% of these cells were macrophages, less than 0.6% were PMNs, and less than 0.35% were lymphocytes (Table 1).

DISCUSSION

The use of modified scavengers of active oxygen species has been proposed for the treatment of a number of diseases, including inflammatory arthritis,[14] ischemia/reperfusion injury,[15] and respiratory distress syndrome.[16] In our work here we sought a preventive approach to asbestos-induced pulmonary fibrosis, a progressive debilitating disease characterized by excessive collagen accumulation in the lung.

Several observations suggest that asbestos-associated cell injury is mediated by active oxygen species. For example, toxicity caused by asbestos in cultures of tracheal epithelial cells, lung fibroblasts, and alveolar macrophages can be prevented by the addition of scavenger enzymes.[3-5] SOD levels are increased in these cell types for as long as 96 h after exposure to asbestos but not after addition of a variety of other dusts.[3,17] Asbestos also causes the generation of superoxide (O_2^-) from alveolar macrophages[18] and the production of O_2^- and OH· from H_2O_2 in a cell-free in vitro system.[19] Since both the generation of free radicals from H_2O_2[19] and asbestos-induced lipid peroxidation[20] are inhibited after incubation of fibers with desferroxamine, the iron content of asbestos appears to be causally related to its catalytic properties.

To determine whether the production of active oxygen species is intrinsic to the pathogenesis of asbestos-induced disease, we have developed a rapid-onset inhalation model of asbestosis[6] and are attempting to

Table 1. Differential Cell Counts in Lavages from 344 Fischer Rats Receiving PEG-catalase

Group[a]	Macrophages	PMNs	Lymphocytes
Sham	99.2 ± 0 15[b]	0.51 ± 0 20	0.33 ± 0 06
PEG-catalase (8×10^2)[c]	99 4 ± 0.10	0 49 ± 0.08	0 06 ± 0 02
PEG-catalase (4×10^3)	99.8 ± 0 10	0 14 ± 0 06	0 07 ± 0 04
PEG-catalase (8×10^3)	99 1 ± 0.20	0 52 ± 0 20	0 30 ± 0 01

[a]N = 4 rats per group Over 1,000 cells from each animal were classified
[b]Represents percentage of total cells (mean ± SE)
[c]IU catalase/rat/day

increase scavenger enzyme levels in the sera and lungs of these animals in an effort to prevent disease. Results here suggest that the administration of PEG-catalase to animals in osmotic pumps is a feasible approach. In initial studies we examined by histology the lungs of normal rats receiving PEG-SOD or PEG-catalase. These sections did not exhibit inflammatory or fibrotic changes (unpublished data). Work here confirms these findings using quantitative biochemical and morphologic parameters of lung injury (LDH, alkaline phosphatase, protein in lavage fluid), inflammation (total and differential cell counts on lavaged cells), and fibrosis (hydroxyproline in lung). In summary, the administration of PEG-catalase for a 28-day period at concentrations of as much as 8,000-IU/day does not appear to perturb normal lung or induce inflammation in rats.

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