Mechanism of Asbestos-mediated DNA Damage: Role of Heme and Heme Proteins

Qamar Rahman,1 Nayyara Mahmood,1 Sikander G. Khan,2 Jamal M. Aref,3 and Mohammad Athar4

1Industrial Toxicology Research Centre, Lucknow, India; 2National Institutes of Health, Bethesda, Maryland; 3Department of Preventive Medicine and Environmental Health, University of Kentucky, Lexington, Kentucky; 4Department of Medical Elementology and Toxicology, Hamdard University, New Delhi, India

Several observations, including studies from this laboratory, demonstrate that asbestos generates free radicals in the biological system that may play a role in the manifestation of asbestos-related cytotoxicity and carcinogenicity. It has also been demonstrated that iron associated with asbestos plays an important role in the asbestos-mediated generation of reactive oxygen species. Exposure to asbestos leads to degradation of heme proteins such as cytochrome P450-releasing heme in cytosol. Our simulation experiments in the presence of heme show that such asbestos-released heme may increase lipid peroxidation and can cause DNA damage. Further, heme and horseradish peroxidase (HRP) can cause extensive DNA damage in the presence of asbestos and hydrogen peroxide/organic peroxide/hydroperoxides. HRP catalyzes oxidation reactions in a manner similar to that of prostaglandin H synthetase. Iron released from asbestos is only partially responsible for DNA damage. However, our studies indicate that DNA damage mediated by asbestos in vivo may be caused by a combination of effects such as the release and participation of iron, heme, and heme moiety of prostaglandin H synthetase in free radical generation from peroxides and hydroperoxides. — Environ Health Perspect 105(Suppl 5):1109–1112 (1997)

Key words: asbestos–DNA, heme, heme protein, heme peroxides

Introduction

Asbestos, a solid-phase carcinogen, causes pleural and peritoneal mesothelioma and bronchogenic carcinoma. The exact mechanism(s) by which asbestos causes these effects has not been fully defined (1).

Several observations, including studies from our laboratory, show that asbestos generates free radicals in the biological system that may play a role in the manifestation of asbestos-related cytotoxicity and carcinogenicity (2). Incubation of asbestos with phagocytes generates oxygen free radicals (3,4). Cultured mesothelial cells are ten times more sensitive than normal human bronchial epithelial cells to toxic effects of asbestos fiber (5); however, relative toxicity of oxygen radicals to the two systems is not known. Asbestos produces DNA damage that has been characterized by increased chromosomal aberrations, 8-hydroxy-2'-deoxyguanosine formation, and increased DNA fidelity, as measured by melting temperature profiles (6–8). We have recently shown that in a predisposed situation of augmented peroxide formation, asbestos exposure further enhances oxidative DNA damage (2). It has also been shown that iron associated with asbestos plays an important role in generation of asbestos-mediated reactive oxygen species (ROS) (9). Iron released from asbestos acting as Fenton’s reagent catalyzes free radical formation (4). In this study we propose that iron released from asbestos under in vivo conditions may not be the sole agent responsible for producing oxidative modifications in DNA but heme may produce similar effects as can be observed by the redox active iron. Alternatively, heme may produce similar effects as can be observed by the redox active iron. In previous studies, we have shown that exposure of asbestos leads to degradation of heme protein and release of heme by cytochrome P450 with the concomitant increase in lipid peroxidation. Heme catalyzes formation of free radicals from organic peroxides and hydroperoxides (2).

In this study, we show that heme and heme protein can cause extensive DNA damage in the presence of asbestos. We observed the involvement of heme-catalyzed ROS by using various ROS quenchers. Our results suggest that heme may be an important factor in oxidative DNA injury during asbestos-mediated carcinogenesis.

Materials and Methods

Chemicals

Calf thymus DNA (sodium salt, average molecular weight of one million) was obtained from Sigma Chemicals (St. Louis, MO) and used without further purification. S1 nuclease was purchased from Bethesda Laboratories (Bethesda, MD). Hydrogen peroxide (H2O2), benzoyl peroxide (BOOB), tertiary-buty1 hydroperoxide (tert-BOOH), cumene hydroperoxide (COOH), 2,2-azo-bis-(isobutryronitrile) (ABIN), and all other chemicals or reagents used were of analytical grade and were purchased from Sigma Research Laboratory (Bombay, India) or Spectrochem Pvt (Bombay, India).

Dust

Union Internationale Contre le Cancer chrysotile and crocidolite reference asbestos samples of 30 µm particle size were a gift from J.B. Leinweber (John Manville Mills, CA). Indian chrysotile mined at Cuddapah, Andhra Pradesh, India, was obtained from Andhra Pradesh Mining Corporation (Hyderabad, India). Indian chrysotile fiber size (30 μm) was prepared according to the procedure followed by Zaidi (10).

Experimental Protocol

Preparation of DNA. A stock solution of DNA (2 mg/ml) was prepared by dissolving
in an appropriate volume of tris-HCl buffer (1 mM, pH 7.5). NaCl (10 mM), and EDTA (2×10⁻⁴ M) (TNE).

Isolation and Treatment of Microsomes with Dust. Rat lung microsomes were obtained according to the procedure described by Rahman et al. (11). Microsomes were incubated with dust samples (1 mg/ml) at 37°C for 20 hr with occasional shaking in a water bath. After incubation, 2.5 ml phosphate buffer (100 mM, pH 7.4) was added. The samples were centrifuged at 105,000×g for 60 min in an ultracentrifuge and the supernatant obtained used as described in "Incubation System."

Incubation System

DNA (1 mg/ml) in a total volume of 2 ml was incubated with crocidolite (1 mg/ml) along with the heme (50 μm), horseradish peroxidase (HRP) (100–200 units), and degradation products of dust-treated and dust-untreated lung microsomes of rats and different peroxides/hydroperoxides—namely H₂O₂, BOO, COOHs, tert-BOOHs, and ABINs—at a final concentration of 40 mM in phosphate buffer (100 mM, pH 7.4). Various peroxides and hydroperoxides were chosen in this study because they mimic the lipid peroxides and hydroperoxides that are generated during excessive lipid peroxidation in lung microsome experiments. Dust was added separately at the time of incubation because microsomes were already pretreated with asbestos as described above.

Assay of S₁ Nuclease Activity. The activity of S₁ nuclease was assayed by estimating the acid-soluble nucleotide released from DNA as a result of enzymatic digestion. The reaction mixture (final volume, 1 ml) contained 600 μg of substrate (native, denatured, or dust, and peroxide-treated DNA) in acetate buffer (100 mM, pH 4.5) containing ZnSO₄ (1 mM) and enzyme S₁ nuclease (100 μl). The reaction mixture was incubated at 37°C for 2 hr. At the end of the incubation period, the reaction was terminated by adding 0.2 ml of bovine serum albumin (10 mg/ml) (mixed thoroughly by shaking and followed by 1 ml of ice-cold 14% perchloric acid). The reaction mixture was kept for 1 hr at 4°C and centrifuged to remove the precipitated protein and undigested DNA.

Results

Like iron, several iron-containing proteins such as heme and those that contain heme as a prosthetic group, e.g., prostaglandin H synthetase, catalyze the formation of free radicals from peroxides (2). Therefore, we demonstrated the effect of HRP, a heme-containing enzyme similar to prostaglandin H synthetase, on the asbestos- and peroxide-mediated degradation of DNA. The peroxides mimic lipid peroxides, which may be generated in excess in a tissue under conditions of oxidative stress. As shown in Table 1, HRP significantly augments asbestos-mediated DNA damage in the presence of hydroperoxides and peroxides. Maximum damage was observed in the presence of H₂O₂. All other peroxides/hydroperoxides appeared to cause much less damage. The presence of hydrogen peroxide increased damage to sugar moiety appreciably, and this was further increased with the addition of HRP. Other peroxides, however, did not produce sugar damage, as shown in Table 2.

To further evaluate whether the heme liberated from degradation of microsomes following asbestos treatment had similar damaging effect on DNA as observed in the presence of purified heme, further studies were conducted. The data for heme alone are not shown. The results of these studies are tabulated in Tables 3 and 4. Heme liberated from microsomal preparations enhanced DNA degradation, which was similar to results observed using purified heme. As shown in Table 3, maximum damage was observed in the presence of H₂O₂. H₂O₂ and ABIN were the only peroxides that caused damage to the sugar moiety of the DNA, as shown in Table 4.

Discussion

It has been proposed that the ROS generated by a fiber directly or by activated phagocytic cells acts as a second messenger to elaborate the cytotoxicity and carcinogenicity of asbestos. Iron plays an important role in ROS generation. Surface characteristics of asbestos fibers and the presence of contaminating iron determine the toxic potential

Table 3. Dose-dependent effect of horseradish peroxidase on the S₁ nuclease hydrolysis of crocidolite-treated DNA in the presence of various peroxides and hydroperoxides.

| Incubation system | DNA hydrolyzed, % | TBA reactive products formed after 20 hr incubation at 37°C, nmol |
|-------------------|------------------|---------------------------------------------------------------|
|                  | Controls         | + H₂O₂ | + COOH | + tert-BOOH | + BOOH | + ABIN | DNA | DNA + HRP | DNA + crocidolite | DNA + crocidolite + HRP | DNA + H₂O₂ | DNA + crocidolite + H₂O₂ | DNA + crocidolite + H₂O₂ + HRP |
| DNA               | 1.30±0.06        | 3.10±0.16 | 2.90±0.88 | 1.82±0.09 | 1.20±0.04 | 3.50±0.19 | DNA | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| DNA + crocidolite | 1.20±0.05        | 40.54±2.10 | 33.58±1.88 | 18.95±1.89 | 24.50±1.47 | 26.92±1.85 | DNA | 3.42±0.27 | 5.34±0.36 |
| DNA + HRP (100 U) | 2.80±0.14        | - | - | - | - | - | DNA + crocidolite | 2.71 |
| DNA + HRP (200 U) | 2.70±0.18        | - | - | - | - | - | DNA + crocidolite | 2.71 |
| DNA + crocidolite + HRP (100 U) | 2.80±0.14 | 54.7±2.18 | 42.18±2.00 | 25.56±2.10* | 29.16±1.82* | 35.65±2.71 |
| DNA + crocidolite + HRP (200 U) | 3.04±0.13* | 67.34±3.20* | 50.05±2.25* | 35.29±1.76* | 39.61±2.20* | 44.28±2.36 |

* p<0.001 when compared to DNA alone or DNA + crocidolite alone. The data represent mean ± SE (n=6). The treatment protocols of DNA samples with crocidolite in presence of H₂O₂ and HRP followed by determination of TBA-reacting species are given in the text.
HEMEmune ASBESTOS-MEDTED DNA DAMAGE

Table 3. Effect of peroxides and hydroperoxides on $S_1$ nuclease hydrolysis of DNA treated with crocidolite-induced degradation products of lung microsomes.

| Incubation system                  | DNA hydrolyzed, % |
|------------------------------------|-------------------|
|                                    | Controls  | $H_2O_2$ | COOH  | tert-BOOH | BOOB | ABIN |
| DNA                               | 1.44±0.07  | 6.00±0.39 | 5.30±0.30 | 4.90±0.29 | 4.50±0.27 | 6.08±0.45 |
| DNA + untreated microsomes        | 2.16±0.14  | 6.12±0.34 | 3.24±0.21 | 1.72±0.11 | 3.60±0.21 | 5.04±0.45 |
| DNA + crocidolite-treated microsomes | 8.68±0.54* | 86.05±6.02* | 28.08±2.24* | 8.64±0.59* | 21.24±1.27* | 23.04±1.38 |

*p<0.001 when compared to DNA alone or DNA + untreated microsomes. The data represent mean ± SE (n=6). The treatment protocols of DNA samples with untreated and crocidolite-treated microsomes in the presence of various peroxides and hydroperoxides are given in the text.

Table 4. Effect of peroxides and hydroperoxides on damage to the deoxyribose sugar moiety of DNA by crocidolite-induced degradation products of lung microsomes.

| Incubation system                  | DNA hydrolyzed, % |
|------------------------------------|-------------------|
|                                    | Controls  | $H_2O_2$ | COOH  | tert-BOOH | BOOB | ABIN |
| DNA                               | 0.00      | 0.00    | 0.00  | 0.00    | 0.00 | 0.00 |
| DNA + untreated microsomes        | 0.00      | 0.00    | 0.00  | 0.00    | 0.00 | 0.00 |
| DNA + crocidolite-treated microsomes | 0.00     | 1.92±0.09 | 0.00  | 0.00    | 1.08±0.07 |

The data represent mean ± SE (n=6). The treatment protocols of DNA samples with untreated and crocidolite-treated microsomes in presence of various peroxides and hydroperoxides are given in the text.

of the fiber (14). Iron acts as a promoter in experimental chemical carcinogenesis and in the development of human neoplasm, as is also concluded in a number of epidemiological studies (4). In light of these observations, the high carcinogenicity of the iron-rich variety of asbestos, crocidolite, compared to that of the low iron variety, chrysotile, is explained. Cationic iron plays a major role in fiber particle uptake by tracheobronchial epithelia and the uptake of fiber particles is also related to iron-mediated enhancement of lipid peroxidation. However, the mechanism through which free radicals are generated by asbestos fibers may not be dependent solely on leachable iron present in asbestos. In our previous study, it was shown that asbestos exposure leads to the degradation of heme proteins such as cytochrome P450, with concomitant release of heme into the system (11). Heme also catalyzes free radical generation from peroxides and hydroperoxides. More recently, we demonstrated that asbestos-mediated DNA damage is enhanced in the presence of peroxides and hydroperoxides. Therefore, the role of heme in catalyzing asbestos and peroxide-mediated damage to DNA was evaluated as a putative mechanism for in vivo DNA damage following asbestos exposure. Heme accumulation in the nucleus has been suggested to be a transacting factor for the transcriptional regulation of heme proteins. However, under the oxidative tone, implications for the accumulation of heme in the nucleus may be dangerous. Results of this study demonstrate the augmentation effect of heme on asbestos- and peroxide-mediated DNA damage. To simulate asbestos-mediated DNA damage in the in vivo situations, experiments were conducted on DNA damage in the presence of microsomes treated with asbestos. Microsomes contain heme protein and cytochrome P450 and release heme on their degradation. Results of these studies indicated augmentation of DNA damage as measured by DNA strand breaks and oxidation of deoxyribose sugar moiety in a fashion similar to that observed with purified heme (data not shown). To further elucidate the role of heme in catalyzing asbestos-mediated DNA damage, the reactions were carried out in the presence of a heme protein, HRP. HRP catalyzes oxidation reactions in a manner similar to that of prostaglandin H synthetase. The prosthetic group of HRP is similar to the heme prosthetic group of prostaglandin H synthetase and both the proteins catalyze two-electron reduction of hydroperoxides. Prostaglandin H synthetase is widely distributed in mammalian tissues and is an abundant accessory protein located mainly in the endoplasmic reticulum and nuclear membranes. Results of this study suggest that the presence of HRP exacerbates DNA damage, supporting the hypothesis that free radicals generated as a result of peroxide reduction by the heme prosthetic group of HRP may be involved in asbestos-induced DNA damage. The heme-catalyzed DNA damage was similar to that catalyzed by HRP, but the magnitude of the damage was much higher in HRP-catalyzed reaction. These observations suggest that asbestos-mediated DNA damage leading to mesothelioma may involve a nuclear heme pool as well as prostaglandin H synthetase or a similar heme containing proteins in the many reactions responsible for generation of DNA-damaging reactive oxygen metabolites.

Experiment results presented in this study suggest that leachable iron from asbestos is responsible for DNA damage in part as desferal, a specific chelator of iron only partially able to suppress DNA damage (data not shown). It is proposed, therefore, that DNA damage mediated by asbestos in vivo may be attributable to a combination of effects that include the release of heme and the participation of heme and a heme moiety of prostaglandin H synthetase in catalyzing free radical generation from peroxides.

In conclusion, our studies indicate that asbestos by generating ROS through iron- and/or heme- and/or prostaglandin H synthetase-catalyzed decomposition of peroxides and hydroperoxides may cause DNA damage. Unlike other carcinogens that produce malignant transformations either by binding to or alkylating DNA, asbestos may act through a different mechanism.

REFERENCES

1. Mossman BT. Medical progress: asbestos-related diseases. N Engl J Med 320:1721-1730 (1989).
2. Mahmood N, Khan SG, Athar M, Rahman Q. Differential role of hydrogen peroxide and organic peroxides in augmenting asbestos-mediated DNA damage: implications of asbestos-induced carcinogenesis. Biochem Biophys Res Comm 200:687-694 (1994).
3. Hatch GE, Gardner DE, Menzel DE. Stimulation of oxidant
production in alveolar macrophages by pollutants and latex particles. Environ Res 23:121–133 (1980).

4. Kamp DW, Graceffa P, Pryor WA, Weitzman SA. The role of free radicals in asbestos-induced diseases. Free Radic Biol Med 12:293–315 (1992).

5. Lechner JF, Tokiwa T, La Veck M, Benedict WF, Banks-Schlegel S, Yeager H Jr, Banerjee A, Harris CC. Asbestos-associated chromosomal changes in human mesothelial cells. Proc Natl Acad Sci USA 82(11):3884–3888 (1985).

6. Adachi S, Kawamura K, Yoshida S, Takemoto K. Oxidative damage on DNA induced by asbestos and man-made fibers in vitro. Int Arch Occup Environ Health 63:553–557 (1992).

7. Jaurand M-C, Kheuang L, Mange L, Bignon J. Chromosomal changes induced by chrysotile fibers of benzo-3,4-pyrene in rat pleural mesothelial cells. Mutat Res 169:141–148 (1986).

8. Takeuchi T, Morimoto K. Crocidolite asbestos increased 8-hydroxydeoxyguanosine levels in cellular DNA of a human promyelocytic leukemia cell line, HL60. Carcinogenesis 15:635–639 (1994).

9. Aust AE. The role of iron in asbestos induced cancer. North Atlantic Treaty Organization Advanced Study Institute Ser H Cell Biol 85:53–61 (1994).

10. Zaidi SH. Experimental pneumoconiosis. Baltimore, MD/London: The Johns Hopkins Press, 1969:33.

11. Rahman Q, Khan SG, Ali S. Effect of chrysotile asbestos on cytochrome P-450-dependent monooxygenase and glutathione S-transferase activities in rat lung. Chem Biol Interact 75:305–314 (1990).

12. Schneider WC. Determination of nucleic acids in tissues by pentose analysis. In: Methods in Enzymology, Vol 3 (Colomic SP, Kaplan ON, eds). New York: Academic Press, 1957:680–684.

13. Gutteridge JMC, Wilkins S. Copper-salt dependent hydroxyl radical formation: damage to proteins acting as antioxidants. Biochem Biophys Acta 759:38–41 (1983).

14. Lung LG, Aust AE. Iron mobilization from crocidolite-dependent formation of DNA single strand breaks in \( \phi \)X174 RF1 DNA. Carcinogenesis 13:637–642 (1992).