Role of Oxidants in DNA Damage

Hydroxyl Radical Mediates the Synergistic DNA Damaging Effects of Asbestos and Cigarette Smoke

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Abstract

The mechanism by which cigarette smoking and asbestos exposure synergistically increase the incidence of lung cancer is unknown. We hypothesized that cigarette smoke and asbestos might synergistically increase DNA damage. To test this hypothesis we exposed isolated bacteriophage PM2 DNA to cigarette smoke and/or asbestos, and assessed DNA strand breaks as an index of DNA damage. Our results supported our hypothesis. 78±12% of the DNA exposed to both cigarette smoke and asbestos developed strand breaks, while only 9.8±7.0 or 4.3±3.3% of the DNA exposed to cigarette smoke or asbestos, respectively, developed strand breaks under the conditions of the experiment.

Our experimental evidence suggested that cigarette smoke and asbestos synergistically increased DNA damage by stimulating ·OH formation. First, significant amounts of ·OH were detected by electron paramagnetic resonance (EPR) in DNA mixtures containing both cigarette smoke and asbestos, but no ·OH was detected in mixtures containing cigarette smoke alone or asbestos alone. Second, the ·OH scavengers, dimethylsulfoxide (DMSO), mannitol, or Na benzoate decreased both ·OH detection by EPR and strand breaks in DNA mixtures exposed to cigarette smoke and asbestos. Third, the H_2O_2 scavenger, catalase, and the iron chelators, 1,10-phenanthroline and desferrithiocin, decreased both ·OH detection and strand breaks in DNA mixtures exposed to cigarette smoke and asbestos. These latter findings suggest that iron contained in asbestos may catalyze the formation of ·OH from H_2O_2 generated by cigarette smoke.

In summary, our study indicates that cigarette smoke and asbestos synergistically increase DNA damage and suggests that this synergism may involve ·OH production.

Introduction

Numerous studies have demonstrated that oxidants cause DNA damage, and it has been speculated that this DNA damage could ultimately lead to carcinogenesis (1–6). Furthermore, since a recent study demonstrated that cigarette smoke generates oxidants (7) and causes DNA damage in cultured cells (8), it has been speculated that oxidants might be responsible for the increased incidence of lung cancer seen among cigarette smokers. Even more striking than the association between cigarette smoking and lung cancer, however, is the association between cigarette smoking, asbestos exposure, and lung cancer. Specifically, asbestos exposed cigarette smokers have a 50–90 times greater incidence of lung cancer (9) while asbestos exposed nonsmokers and non-asbestos–exposed cigarette smokers have only a 5 and 10 times, respectively, greater incidence of lung cancer than non-asbestos–exposed, nonsmoking individuals (9, 10). The mechanism of this synergy between cigarette smoking and asbestos exposure is unknown. Previous studies have indicated, however, that cigarette smoke generates superoxide anion ($O_2^{\cdot}$) and hydrogen peroxide (H_2O_2; 7), and that iron stimulates the production of hydroxyl radical (·OH) from $O_2^{\cdot}$ and H_2O_2 (11). Since ·OH is the specific oxidant thought to be responsible for cigarette smoke–mediated DNA damage (8), and because asbestos contains a large amount of iron and can stimulate ·OH production from H_2O_2 (12), we hypothesized that asbestos would synergistically increase the amount of damage seen in DNA exposed to cigarette smoke and that this increased damage might be due to stimulation of ·OH formation. In order to test this hypothesis we exposed isolated DNA to cigarette smoke, asbestos, ·OH scavengers, and/or iron chelators, and assessed the degree of DNA damage and ·OH production in our reaction mixtures.

Methods

Preparation of smoke phosphate-buffered saline (PBS). Smoke PBS was prepared according to the method of Nagata et al. (7). Briefly, smoke from one commercial filter cigarette was bubbled through 6 ml of PBS, pH 7.4 for 5 min. 10 μl of this smoke-PBS was then added to reaction mixtures, as outlined below.

Measurement of DNA damage. DNA strand breaks were measured according to a modification of the method of Lown (13). Briefly, this assay detects strand breaks in closed covalently circular (CCC)[1] DNA by assessing the degree of ethidium bromide fluorescence in DNA mixtures exposed to alkaline and heat denaturing conditions. When double stranded, CCC PM2 DNA develops strand breaks, it is converted into open circular (OC) or linear DNA. OC and linear DNA become single stranded when exposed to heat and alkaline denaturing conditions, but CCC DNA (which contains no strand breaks) remains double stranded under these same conditions. Since ethidium bromide preferentially binds to double stranded DNA, one can assess the relative amounts of nonbroken, double stranded, CCC DNA and broken, OC or linear DNA, by measuring the degree of ethidium bromide fluorescence in reaction mixtures.

Reaction mixtures for measurement of DNA damage consisted of 0.8 μg of PM2 bacteriophage CCC DNA (Boehringer Mannheim, Indianapolis, IN), along with various combinations of 0.05 mg of crocido-

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1. *Abbreviations used in this paper:* CCC, closed covalently circular; DFT, desferrithiocin; DMPO, 5,5-dimethyl-1-pyroline-*N*-oxide; EPR, electron paramagnetic resonance; OC, open circular; PHEN, 1,10-phenanthroline; SOD, superoxide dismutase.

lite asbestos (U.I.C.C., reference standard sample, kindly supplied by Dr. V. Timbrell and Dr. J. C. Wagner, Pneumoconiosis Research Unit; Medical Research Council; Penarth, UK), 10 μl of smoke PBS, 65 μM hydrogen peroxide (Fisher Scientific Co., Inc., Fair Lawn, NJ), 30 μM $FeSO_4$ (Sigma Chemical Co., St. Louis, MO), 100 mM dimethylsulfoxide (DMSO; Sigma Chemical Co.), 100 mM mannitol (Calbiochem-Behring Corp., La Jolla, CA), 100 mM Na benzoate, 4 mM 1,10-phenanthroline (PHEN; both from Sigma), 4 mM desferrithiocin (DFT, kindly supplied by Ciba Geigy Ltd., Basel, Switzerland), 3.5 μg of catalase, and/or 3.5 μg of superoxide dismutase (SOD, both from Sigma) in a final volume of 100 μl of PBS, pH 7.4. Reaction mixtures were incubated for 1 h at room temperature, and were then centrifuged at 10,000 $g \times 10$ s to pellet the asbestos particles. (The asbestos particles interfered with our fluorescence measurements.) Supernatants (50 μl) were transferred to glass tubes containing 500 μl of fluorescence assay solution (0.5 μg/ml ethidium bromide, 0.5 mM EDTA, 20 mM tripotassium phosphate, pH 11.8; all from Sigma). Mixtures were heated at 96°C for 4 min, placed on ice, and equilibrated to 25°C. Fluorescence was measured on a fluorescence spectrophotometer (model 650-15; Perkin-Elmer Corp., Hitachi-Perkin-Elmer Instruments, Mountain View, CA) using an excitation wavelength of 525 and an emission wavelength of 600. All fluorescence values were obtained after heating at 96°C. The percentage of DNA developing strand breaks was calculated according to the formula: % DNA developing strand breaks = [1 − [fluorescence of injured DNA/fluorescence of noninjured DNA]] × 100.

Measurement of ·OH production. ·OH production was detected as previously described (12), using electron paramagnetic resonance (EPR) spectroscopy and the spin trap 5,5-dimethyl-1-pyroline-*N*-oxide (DMPO). DMPO was purified by charcoal filtration according to the method of Buettner et al. (14). Reaction mixtures for measurement of ·OH were identical to reaction mixtures for measurement of DNA damage except for the addition of 100 mM DMPO (Aldrich Chemical Co., Milwaukee, WI) to all reaction mixtures. In addition, 6.5 M ethanol (U.S. Industrial Chemical Co., New York) was added to some of the reaction mixtures, in order to prove that the EPR signal generated was due to ·OH production. Immediately after the addition of all reaction components, mixtures were aspirated into 5-in. glass capillary tubes and inserted into an EPR spectrophotometer (E-109 X-band, Varian Associates, Palo Alto, CA) operating at 100 kHz field modulation, 2 G modulation amplitude, 10 mW microwave power level, 3.2 $\times 10^4$ gain setting, 0.064 second time constant and 50 G/min scan speed. The EPR spectra were single scanned.

Statistical analysis. Results were analyzed using one-way analysis of variance with Student-Newman-Keuls multiple comparison procedure, and two-tailed Student's *t* test.

Results

DNA damage. 9.8±7.0, 4.3±3.3, or 5.3±5.5% of the DNA exposed to cigarette smoke alone, asbestos particles alone, or $FeSO_4$ alone, respectively, developed strand breaks (Fig. 1). In contrast, 78±12 or 86±12% of the DNA exposed to both cigarette smoke and asbestos, or cigarette smoke and $FeSO_4$, respectively, developed strand breaks (Fig. 1). Similarly, 6.5±5.3% of the DNA exposed to H_2O_2 alone developed strand breaks while 24±6.3 or 25±8.1% of the DNA exposed to both H_2O_2 and asbestos or H_2O_2 and $FeSO_4$, respectively, developed strand breaks (Fig. 1).

The ·OH scavengers, DMSO, mannitol, or Na benzoate, the H_2O_2 scavenger, catalase, and the iron chelators, PHEN or DFT all prevented strand breaks in DNA mixtures exposed to cigarette smoke and asbestos (Fig. 2). Specifically, 78±12% of the DNA exposed to cigarette smoke and asbestos developed strand breaks, while only 0, 5.5±6.4, 1.5±3, 4.8±7.1, 4.0±5.7 and 0% of the DNA exposed to cigarette smoke and asbestos in the presence of DMSO, mannitol, Na benzoate, catalase, PHEN, or DFT, respectively, developed strand breaks. The O_2^- scavenger, SOD, or heat inactivated catalase did not decrease DNA strand breaks (data not shown). The fluorescence values of uninjured, control DNA, were not significantly different in the presence or absence of DMSO, mannitol, Na benzoate, catalase, SOD, PHEN, or DFT (data not shown).

·OH production. DNA mixtures containing cigarette smoke and asbestos generated ·OH. Specifically, DNA mixtures containing cigarette smoke, asbestos, and the spin trap, DMPO, generated the characteristic four-line spectrum of the hydroxyl radical adduct of DMPO (DMPO-OH; 15; Fig. 3 *A*). Similarly, DNA mixtures containing either cigarette smoke and $FeSO_4$, H_2O_2 and asbestos, or H_2O_2 and $FeSO_4$ also generated DMPO-OH (Fig. 3 *B, C,* and *D,* respectively). Mixtures containing DNA, cigarette smoke, asbestos, $FeSO_4$, or H_2O_2 alone did not generate detectable amounts of DMPO-OH (Fig. 3 *E, F, G, H,* and *I,* respectively).

When various ·OH scavengers (ethanol, DMSO, mannitol, or Na benzoate), H_2O_2 scavengers (catalase), or iron chelators (PHEN or DFT) were added to DNA mixtures containing cigarette smoke, asbestos, and DMPO, the DMPO-OH signal was either diminished or completely abolished. Specifically,

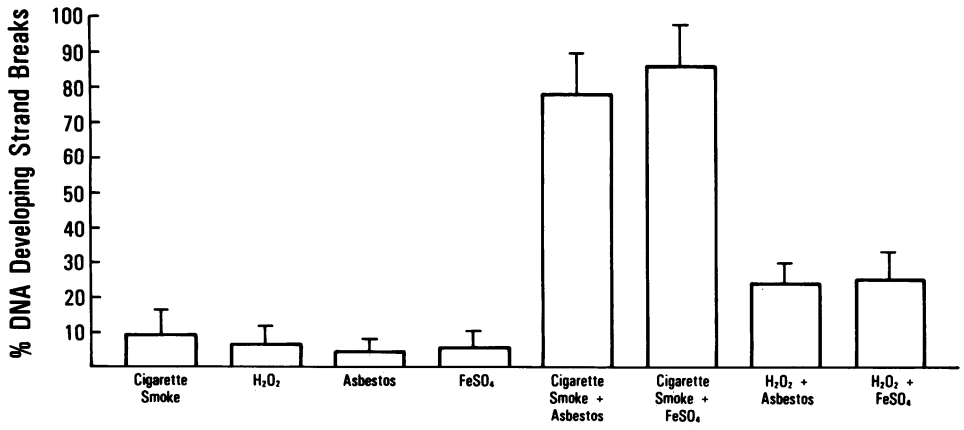

Figure 1. Development of DNA strand breaks after exposure to various agents. Reaction mixtures consisted of 0.8 μg of PM2 DNA, 0.05 mg of crocidolite asbestos, 10 μl of smoke PBS, 65 μM H_2O_2, and/or 30 μM $FeSO_4$ in a final volume of 100 μl of PBS, pH 7.4. Reactions were incubated at room temperature for 60 min. Values are means±SD. Each value represents four separate experiments performed in triplicate. Values for cigarette smoke + asbestos, cigarette smoke + $FeSO_4$, H_2O_2 + asbestos, or H_2O_2 + $FeSO_4$ are significantly greater ($P < 0.05$) than the sums of the values of their respective components.

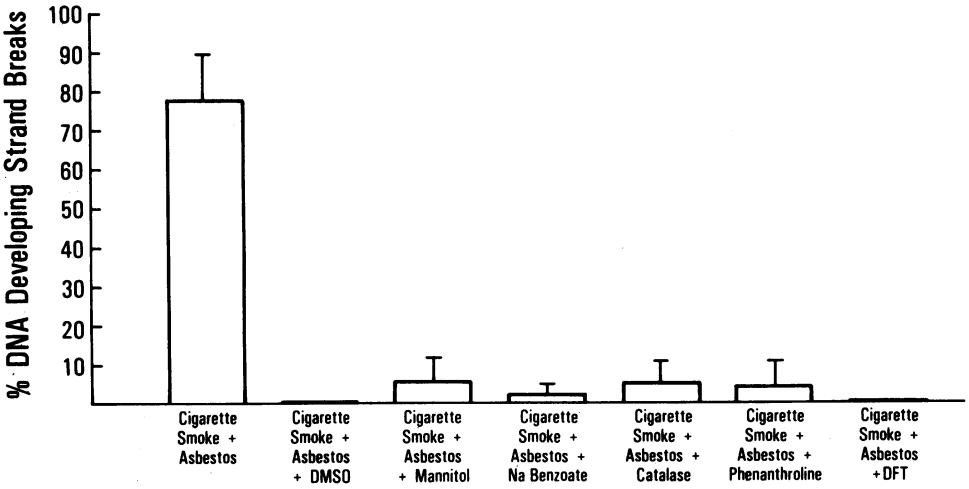

Figure 2. Inhibition of cigarette smoke and asbestos-mediated DNA strand breaks by ·OH scavengers, catalase, or iron chelators. Reaction mixtures and conditions were identical to those in Fig. 1, except for the addition of 100 mM DMSO, 100 mM mannitol, 100 mM Na benzoate, 3.5 μg catalase, 4 mM 1,10-phenanthroline, or 4 mM DFT. Values are means±SD. Each value represents four separate experiments performed in triplicate. Values for mixtures containing DMSO, mannitol, Na benzoate, catalase, PHEN, or DFT are all significantly less ($P < 0.05$) than values for mixtures without these additions.

when ethanol was added to reaction mixtures, the DMPO-OH spectrum was no longer observed and was replaced by the characteristic spectrum of the alpha-hydroxyethyl radical adduct of DMPO (15, Fig. 4 *B*). Similarly, when DMSO was added to reaction mixtures, the DMPO-OH signal was markedly decreased and was replaced in large part by the characteristic signal of the methyl radical adduct of DMPO (DMPO-CH_3; 16; Fig. 4 *C*). Mannitol slightly decreased the magnitude of the DMPO-OH signal (Fig. 4 *D*), Na benzoate significantly decreased the magnitude of the DMPO-OH signal, (Fig. 4 *E*),

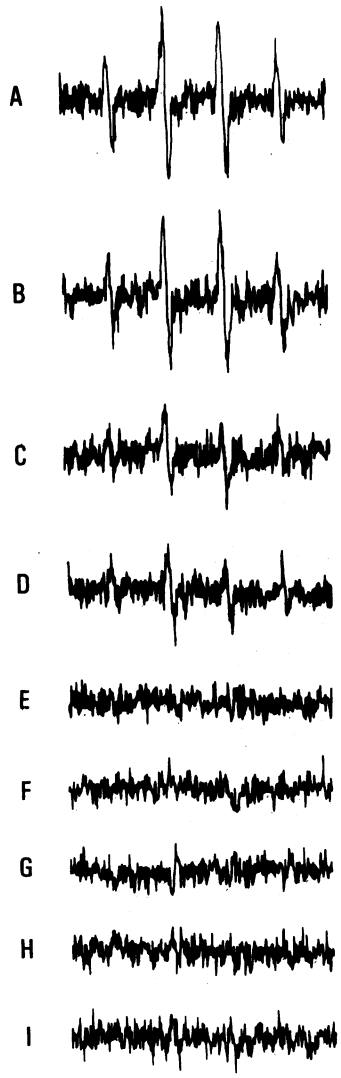

Figure 3. ·OH production in various DNA mixtures. Reaction mixtures are identical to those in Fig. 1, except for the addition of 100 mM DMPO. Tracings are EPR spectra obtained immediately after the addition of all reaction components. (*A*) DNA + cigarette smoke + asbestos, (*B*) DNA + cigarette smoke + $FeSO_4$, (*C*) DNA + H_2O_2 + asbestos, (*D*) DNA + H_2O_2 + $FeSO_4$, (*E*) DNA alone, (*F*) cigarette smoke alone, (*G*) asbestos alone, (*H*) $FeSO_4$ alone, (*I*) H_2O_2 alone.

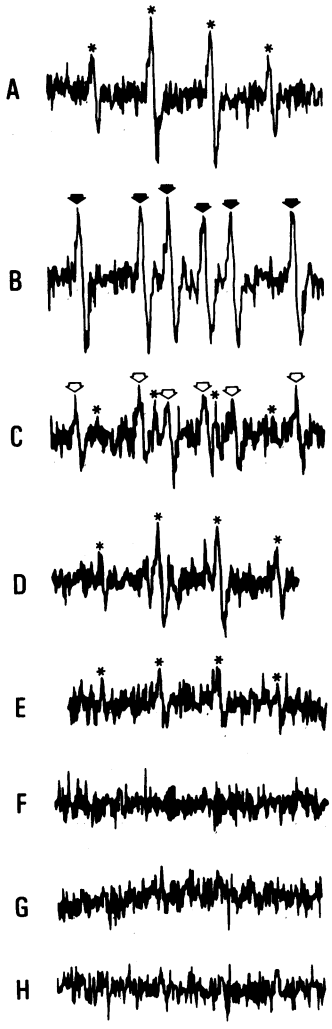

Figure 4. Inhibition of ·OH production by ·OH scavengers, catalase, or iron chelators. Reaction mixtures are identical to those in Fig. 2, except for the addition of 100 mM DMPO. Tracings are EPR spectra of DNA mixtures containing cigarette smoke, asbestos, and the following components: (*A*) no additional components, (*B*) ethanol (6.5 M), (*C*) DMSO, (*D*) mannitol, (*E*) Na benzoate, (*F*) catalase, (*G*) PHEN, (*H*) DFT. (*, DMPO-OH signal; *closed arrows,* DMPO-alpha-hydroxyethyl radical signal; and *open arrows,* DMPO-CH_3 signal.)

and catalase, PHEN, and DFT completely abolished the DMPO-OH signal (Fig. 4 *F–H*, respectively). SOD did not significantly decrease the magnitude of the DMPO-OH signal (data not shown). Ethanol, DMSO, mannitol, Na benzoate, catalase, SOD, PHEN, or DFT alone did not generate detectable EPR signals (data not shown).

Discussion

Our results indicate that asbestos particles synergistically increase the amount of strand breaks in isolated DNA exposed to cigarette smoke. Several lines of evidence suggest that asbestos causes this synergy by stimulating $^{\bullet}OH$ production.

First, the hydroxyl radical adduct of DMPO (DMPO-OH) was detected by EPR in DNA mixtures containing cigarette smoke and asbestos but was not detected in DNA mixtures containing cigarette smoke alone or asbestos alone. Since DMPO-OH can occasionally be artifactually produced through a mechanism that is not dependent on $^{\bullet}OH$ (15), we had to verify that DMPO-OH was specifically due to $^{\bullet}OH$ in our DNA mixtures. The accepted method to perform this verification involves the use of the secondary $^{\bullet}OH$ trap, ethanol. Ethanol reacts with $^{\bullet}OH$ to produce alpha-hydroxyethyl radicals (15), which can then form adducts with DMPO. When, therefore, DMPO-OH formation is due to the spin trapping of $^{\bullet}OH$, ethanol addition inhibits DMPO-OH formation, and causes DMPO-alpha-hydroxyethyl radical formation (15). Since addition of 6.5 M ethanol to our DNA mixtures containing cigarette smoke, asbestos, and DMPO prevented DMPO-OH formation and caused DMPO-alpha-hydroxyethyl radical formation, we conclude that DMPO-OH was specifically due to $^{\bullet}OH$ in our DNA mixtures. This conclusion was further supported by our studies with DMSO. Although the purpose of adding DMSO to our reaction mixtures was to prevent DNA damage, the chemical properties of DMSO also made it useful in confirming $^{\bullet}OH$ production. Since DMSO reacts with $^{\bullet}OH$ to form $^{\bullet}CH_3$, and $^{\bullet}CH_3$ can react with DMPO to form DMPO-CH_3 (15, 16), the formation of DMPO-CH_3 in our DNA mixtures containing DMSO, cigarette smoke, asbestos, and DMPO was further evidence for $^{\bullet}OH$ formation. It should be noted that although we were unable to detect DMPO-OH in our DNA mixtures containing cigarette smoke alone, it is possible that small quantities of $^{\bullet}OH$ were produced but were below the levels detectable by the EPR. Previous investigators (17, 18) using much larger quantities of aqueous extracts of cigarette tar have demonstrated that metals in cigarette tar can stimulate $^{\bullet}OH$ production from H_2O_2 generated by cigarette tar. Furthermore, our observation that 9.8±7.0% of our DNA exposed to cigarette smoke alone developed strand breaks that could be prevented by the $^{\bullet}OH$ scavengers DMSO, mannitol or Na benzoate (100 mM, data not shown), also suggests that small quantities of $^{\bullet}OH$ were generated in our DNA mixtures exposed to cigarette smoke alone.

Second, several different $^{\bullet}OH$ scavengers inhibited the detection of $^{\bullet}OH$ and also prevented strand breaks in DNA mixtures containing cigarette smoke and asbestos. As can be seen in Fig. 4, there was a marked difference between the ability of the various $^{\bullet}OH$ scavengers to inhibit the DMPO-OH signal. (DMSO > Na benzoate > mannitol). Furthermore, although all the $^{\bullet}OH$ scavengers very effectively inhibited DNA strand breaks, none of them completely inhibited the DMPO-OH signal. These apparent discrepancies can be easily explained. Inhibition of DNA damage required that the added scavengers (DMSO, mannitol, Na benzoate) be able to effectively compete with DNA for $^{\bullet}OH$. In contrast, inhibition of the DMPO-OH signal required that the added scavengers be able to effectively compete with DMPO for $^{\bullet}OH$. Moreover, since the velocity of the reaction of DMPO or the various scavengers with $^{\bullet}OH$ depends on their rate constants and molar concentrations, and since the concentration of DMPO in our reaction mixtures was the same as the concentration of the scavengers, one might expect that the ability of the scavengers to compete with DMPO for $^{\bullet}OH$ would correlate with their relative rate constants for reaction with $^{\bullet}OH$. The bimolecular rate constants for the reaction of DMSO, Na benzoate, mannitol, or DMPO with $^{\bullet}OH$ are 7×10^9, $3.3–3.8 \times 10^9$, 1×10^9, and 3.4×10^9 M^{-1} s^{-1}, respectively, (15, 19–21). Because we found that DMSO was the most effective inhibitor of the DMPO-OH signal, whereas mannitol was the least effective inhibitor of the DMPO-OH signal, the ability of the various scavengers to inhibit the DMPO-OH signal appeared to correlate with their bimolecular rate constants for reaction with $^{\bullet}OH$.

Third, the H_2O_2 scavenger, catalase, (but not heat-inactivated catalase) decreased the amount of $^{\bullet}OH$ detected and also decreased the amount of strand breaks in DNA mixtures containing cigarette smoke and asbestos. Cigarette smoke has previously been demonstrated to generate $O_2^{\cdot-}$ and H_2O_2 (7). Specifically, $O_2^{\cdot-}$ and H_2O_2 can be generated from polyphenols (such as catechols, catechol derivatives, benzopyrene metabolites, or hydroquinones) that are present in cigarette smoke (7, 8, 22, 23). Once generated, $O_2^{\cdot-}$ and H_2O_2 can, in the presence of iron, be converted into $^{\bullet}OH$. This conversion is thought to occur via the iron-catalyzed modified Haber-Weiss reaction depicted below:

$$O_2^{\cdot-} + Fe^{3+} \rightarrow O_2 + Fe^{2+}$$

$$H_2O_2 + Fe^{2+} \rightarrow {}^{\bullet}OH + Fe^{3+} + OH^- \quad (11)$$

In the presence of asbestos, the following reaction has been suggested to occur: H_2O_2 + (asbestos)-Fe^{2+} → $^{\bullet}OH$ + (asbestos)-Fe^{3+} (12). It is probable, therefore, that catalase prevented DNA strand breaks by scavenging H_2O_2, thereby preventing its conversion into $^{\bullet}OH$. This observation is important because it suggests that although cigarette smoke contains many components that could potentially interact with asbestos (or iron) and synergistically increase DNA damage, it is likely that H_2O_2 is the component in cigarette smoke that is responsible for this effect. This premise is further supported by the observation that the $O_2^{\cdot-}$ scavenger, SOD, did not decrease the amount of $^{\bullet}OH$ detected or the amount of strand breaks in DNA mixtures containing cigarette smoke and asbestos, and the observation that the addition of reagent H_2O_2 to DNA mixtures containing asbestos or $FeSO_4$ also synergistically increased $^{\bullet}OH$ production and strand breaks. The ability of H_2O_2 and asbestos to damage DNA has also been reported by Kasai et al. (24). These latter findings may suggest that $^{\bullet}OH$ formation in our system could involve Fenton chemistry rather than the modified Haber-Weiss reaction, or that agents in cigarette smoke (other than $O_2^{\cdot-}$) could reduce Fe^{3+} to Fe^{2+}.

Fourth, the iron chelators DFT (25) and PHEN prevented $^{\bullet}OH$ detection and strand breaks in DNA mixtures containing cigarette smoke and asbestos. In the Fenton reaction or modi-

fied Haber-Weiss reaction, iron stimulates the conversion of O_2^- and H_2O_2 into $^{\cdot}OH$. Crocidolite asbestos has been shown, by neutron activation analysis, to contain 27% iron (26). This iron is not a contaminant, but rather is an integral part of the fibrous silicate lattice structure of asbestos (12). Since it is known that certain iron chelators (such as PHEN) can inhibit the ability of iron to catalyze $^{\cdot}OH$ production (27), it is possible that DFT and PHEN prevented $^{\cdot}OH$ detection and strand breaks in DNA mixtures containing cigarette smoke and asbestos by chelating the iron in the asbestos. This premise is supported by the studies of Weitzman et al. (12). Specifically, they demonstrated that asbestos could stimulate $^{\cdot}OH$ production in the presence of H_2O_2, and this $^{\cdot}OH$ production was inhibited by the iron chelator desferroxamine. Because the iron chelators employed in our study are not totally specific for iron, we performed additional studies in which $FeSO_4$ was substituted for asbestos in DNA mixtures containing cigarette smoke. $FeSO_4$ also synergistically increased the amount of $^{\cdot}OH$ detected and the amount of strand breaks in DNA mixtures containing cigarette smoke. It appears, therefore, that although we cannot exclude the participation of other metal contaminants in our system, it is likely that the iron contained in the asbestos particles is responsible for a significant amount of our observed results.

Taken in toto, our results suggest that asbestos may synergistically increase isolated DNA strand breaks by stimulating $^{\cdot}OH$ production from oxidants generated by cigarette smoke.

Several in vivo studies have demonstrated that asbestos fibers can be found within the cytoplasm and/or nucleus of viable alveolar macrophages, type 1 epithelial cells, type II epithelial cells, fibroblasts, and/or endothelial cells after asbestos inhalation (28, 29). Similarly, asbestos fibers have been found within the cytoplasm and/or nucleus of viable fibroblast or macrophages exposed to asbestos in tissue culture (30, 31). Moreover, it has been demonstrated that asbestos can adsorb benzopyrene (as well as other polycyclic aromatic hydrocarbons) onto its surface (32, 33). It appears, therefore, that in addition to being able to reach key intracellular targets, asbestos particles might also facilitate the transport of oxidant generators to these targets. Since intracellular scavengers could significantly decrease or even prevent the diffusion of extracellularly generated oxidants to the nucleus, the potential ability of benzopyrene adsorbed asbestos fibers to deliver both an oxidant generator and a metal catalyst to critical intracellular targets could be important, because it would allow $^{\cdot}OH$ production to occur in close proximity to cellular DNA. Finally, since iron can leach out of asbestos fibers in vitro (data not shown), it is possible that iron contained in extracellular or intracytoplasmic asbestos particles might leach out of the asbestos particles and diffuse or be transported into the nucleus where it also could stimulate $^{\cdot}OH$ production and DNA damage. It appears, therefore, that although the aim of our study was to demonstrate that asbestos and cigarette smoke synergistically increased in vitro DNA damage by stimulating $^{\cdot}OH$ production, a similar mechanism could exist and be relevant in vivo. In addition, since cigarette smoke or asbestos stimulates neutrophils and macrophages to accumulate in the lung, and since neutrophils, macrophages, and tracheal epithelial cells have been demonstrated to release O_2^- and/or H_2O_2 in response to asbestos fibers (34, 35), it is also possible that asbestos may increase DNA damage by stimulating $^{\cdot}OH$ production from cell-derived oxidants.

Recent studies have suggested that DNA strand breaks (or other forms of DNA damage) caused by active oxygen species may be involved in tumor promotion and malignant transformation (1–6). Although it is likely that the majority of DNA damage that occurs in a human body is efficiently repaired, it is possible that some damaged DNA could occasionally either escape repair or be incorrectly repaired. If these errors in repair accumulate over a period of time, it is conceivable that these errors could ultimately contribute to carcinogenesis. Obviously, further studies will be required to elucidate the exact role of DNA damage in carcinogenesis. Nevertheless, the present study suggests that stimulation of $^{\cdot}OH$-mediated DNA damage could help explain the synergistically increased incidence of lung cancer commonly observed in cigarette smokers exposed to significant amounts of asbestos particles.

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