Asbestos-catalysed lipid peroxidation and its inhibition by desferroxamine

Sigmund A. WEITZMAN* and Alan B. WEITBERG

Hematology–Oncology Unit, Massachusetts General Hospital Cancer Center, and Department of Medicine, Harvard Medical School, Boston, MA 02114, U.S.A.

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In an effort to understand the properties of asbestos fibres that might contribute to their being toxic, we incubated three different varieties of asbestos with phospholipid emulsions and looked for evidence of lipid peroxidation. Although all three types of asbestos were able to catalyse lipid peroxidation in the native state, this catalytic activity was inhibited by pre-washing of the asbestos with the iron chelator desferroxamine. This suggests that: (1) lipid peroxidation may be one of the mechanisms by which asbestos produces tissue injury, and (2) treatment with iron chelators might diminish the potential to produce this injury.

The term ‘asbestos’ applies to a group of fibrous silicate minerals sharing certain properties that are commercially and industrially desirable. Asbestos fibres also share certain toxic properties: they can produce inflammation, fibrous scarring and cancer (Craighead & Mossman, 1982; Becklake, 1982). The physical dimensions of the fibres appear to be very important in determining the amount of toxicity produced (Stanton *et al.*, 1981; Hesterberg & Barrett, 1984), but the chemical processes resulting in the toxicity require further elucidation (Harington, 1981; Becklake, 1982; Mossman & Landesman, 1983).

We recently showed (Weitzman & Graceffa, 1984) that asbestos fibres can catalyse the production of hydroxyl radicals from hydrogen peroxide. Others also have suggested that oxygen radicals are important in the pathogenesis of asbestos toxicity (Mossman & Landesman, 1983). We also presented data indicating that the iron content of the asbestos was responsible for much of this catalytic activity (Weitzman & Graceffa, 1984). Since iron compounds or iron chelates have been shown to produce lipid peroxidation, cytotoxicity and tissue damage in certain settings (Crichton, 1979; Bacon *et al.*, 1983; Halliwell & Gutteridge, 1984), we questioned whether asbestos fibres as such were catalytically active enough to accelerate lipid peroxidation under ambient oxygen partial pressure.

If so, this might be one of the mechanisms by which asbestos produces tissue injury.

Methods

Three different types of asbestos were used in these experiments: crocidolite, amosite and Canadian chrysotile. All were U.I.C.C. Reference Standard samples (Timbrell, 1970) kindly supplied by Dr. V. Timbrell and Dr. J. C. Wagner. Each was suspended in deionized distilled water that had been previously treated with Chelex-100 (Bio-Rad Laboratories), 1 vol. to 10 vol. of water, to remove contaminating iron (Gutteridge *et al.*, 1981). All water used in the experiments was similarly pretreated with Chelex-100.

In some experiments the chelator ADP (Sigma Chemical Co.) was added to the asbestos suspensions. In other experiments desferroxamine B (mesylate salt; from Geigy) in various concentrations was added to the stock 5 mg/ml asbestos suspensions. After overnight shaking, the asbestos fibres were sedimented by centrifugation at 600g for 15 min and resuspended in water. This wash procedure (centrifugation followed by resuspending in water) was done three times and the asbestos was resuspended in water.

Phospholipid emulsions were prepared from dried asolectin (Associated Concentrates, Woodside, NY, U.S.A.), a mixture of soya-bean phosphatides (Chan *et al.*, 1982). This material was prepared freshly for each experiment by grinding with a mortar and pestle into a powder, and suspended in deionized water at a concentration of

* To whom correspondence should be addressed, at: Hematology–Oncology Unit, Cox Building, 6th Floor, Massachusetts General Hospital, Boston, MA 02114, U.S.A.

5mg/ml. The suspension was then sonicated for 5min in a water-bath sonifier and used in the experiments promptly thereafter.

Lipid peroxidation was measured by the thiobarbituric acid method. This method detects products of lipid peroxidation, particularly malondialdehyde and its precursors, but also including other lipid oxidation products, and is a reliable method for determining the extent of lipid peroxidation *in vitro* (Buege & Aust, 1978). Experimental incubations included asbestos (final concn. 500 μ g/ml) and asolectin (5mg/ml), with or without chelators, to a total volume of 1.0ml. The tubes were then immersed in a 37°C water bath in room air for various times. To stop the reaction, the tubes were placed promptly on ice, and catalase (from bovine liver, 33900 units/mg; Sigma Chemical Co.) (final concn. 0.1mg/ml) and butylated hydroxytoluene (Sigma Chemical Co.) (final concn. 1 mM) were added immediately (Chan *et al.*, 1982). To this tube was added 2ml of a freshly prepared solution containing trichloroacetic acid (15%, w/v), thiobarbituric acid (0.375%) and 0.25M-HCl (thiobarbituric acid reagent). After mixing, the tubes were plugged with cotton, heated for 15min in a boiling-water bath, cooled on ice for 5min and centrifuged at 1000g for 10min. Samples were then assayed for absorbance at 535nm, against a blank containing the thiobarbituric acid reagent. Concentrations of thiobarbituric acid-reactive material in the samples were determined by comparison with standard curves generated by addition of measured amount of 1,1,3,3-tetraethoxypropane (Sigma Chemical Co.) to the thiobarbituric acid reagent and heating in a boiling-water bath as above.

Results

Each of the three types of asbestos was capable of causing a significant lipid peroxidation by 15min. Although all three were active, each was significantly different from the other (Fig. 1), with Canadian chrysotile causing the most peroxidation, crocidolite an intermediate amount, and amosite the least. Little change was noted in the control incubations, which consisted of asolectin without added asbestos.

The effect of the presence of 1 mM-ADP in the incubations is illustrated in Table 1. There is marked augmentation of peroxidation in the cases of both chrysotile and amosite. In contrast, ADP had an inhibitory effect on peroxidation produced by crocidolite.

The effects of washing the asbestos with different concentrations of the iron chelator desferrioxamine are also illustrated in Table 1. In each case the final concentration of asbestos was

Fig. 1. Effects of amosite, crocidolite and Canadian chrysotile asbestos on lipid peroxidation in suspensions of soya-bean phosphatides (asolectin)
●, Control (no asbestos); ○, amosite; △, crocidolite; □, chrysotile.

Table 1. Effects of desferrioxamine (DEF) and ADP on asbestos-mediated lipid peroxidation

Incubations were performed for 15min at 37°C. Numbers in parentheses indicate numbers of determinations. Results are expressed as concentration of thiobarbituric acid-reactive material in the incubation, mean $\pm$ S.E.M.

Incubation conditions	Thiobarbituric acid-reactive material (μ M)
Asolectin control (60)	0.360 $\pm$ 0.02
Asolectin + chrysotile (40)	1.00 $\pm$ 0.06
Asolectin + chrysotile + 1 mM-DEF (21)	0.750 $\pm$ 0.08
Asolectin + chrysotile + 5 mM-DEF (12)	0.575 $\pm$ 0.05
Asolectin + chrysotile + 10 mM-DEF (12)	0.510 $\pm$ 0.05
Asolectin + chrysotile + 1 mM-ADP (12)	2.01 $\pm$ 0.26
Asolectin + crocidolite (19)	0.690 $\pm$ 0.03
Asolectin + crocidolite + 1 mM-DEF (3)	0.485 $\pm$ 0.02
Asolectin + crocidolite + 5 mM-DEF (3)	0.355 $\pm$ 0.02
Asolectin + crocidolite + 1 mM-ADP (6)	0.352 $\pm$ 0.03
Asolectin + amosite (16)	0.535 $\pm$ 0.04
Asolectin + amosite + 1 mM-DEF (3)	0.325 $\pm$ 0.02
Asolectin + amosite + 1 mM-ADP (5)	0.850 $\pm$ 0.02

500 μ g/ml. Desferrioxamine washing was able to decrease the lipid peroxidation produced by each of the three types of asbestos. Amosite-catalysed peroxidation was most sensitive to inhibition by desferrioxamine, that with crocidolite intermedi-

ate, and that with chrysotile least sensitive. Inhibition of peroxidation was also observed when desferroxamine was added (in these same concentrations) to the incubations simultaneously with the asbestos and asolectin.

Discussion

The major points made in this study are: (1) different forms of asbestos fibres share the property of being able to catalyse lipid peroxidation *in vitro*, (2) the iron content of the asbestos appears to be responsible for much of this activity, and (3) washing asbestos with desferroxamine inhibits the catalysis.

Lipid peroxidation may be toxic by a variety of mechanisms. These include alterations in membrane fluidity (Chiu *et al.* 1982), initiation of radical chain reactions (Tappel, 1973; Pietronigro *et al.*, 1977; McKay 1981), direct toxicity of lipid breakdown products (Mukai & Goldstein, 1976; Bird *et al.*, 1982; Esterbauer, 1982; Morel *et al.*, 1983) and effects on intermediate metabolism of toxins (Dix & Marnett, 1983). Thus the finding that asbestos can mediate lipid peroxidation suggests several chemical mechanisms, in addition to the previously described generation of oxygen radicals, by which these inorganic fibres may be toxic.

On the basis of our previous studies, we suspected that the iron content of the asbestos would be involved in the pathogenesis of peroxidation. All three varieties tested contain substantial amounts of iron (amosite 28%, crocidolite 27%, and Canadian chrysotile 2.6%, as determined by neutron activation analysis) (Timbrell, 1970). Whereas iron forms a major part of the amphibole (crocidolite and amosite) crystal structure, the ideal chemical composition of the serpentine asbestos, chrysotile, is $\text{Mg}_3\text{Si}_2\text{O}_5(\text{OH})_4$. Other metals, however, can substitute for the magnesium, with iron being predominant (Pooley, 1981). Furthermore, iron and other metals can contaminate the surface of the fibres. Since excess tissue iron can cause lipid peroxidation, cellular disruption and fibrosis in the liver and other organs (Crichton, 1979; Bacon *et al.*, 1983), it seemed reasonable to suspect that this might play a role in asbestos injury in the lung. The efficacy of desferroxamine, a chelator that can neutralize the catalytic activity of iron (Gutteridge *et al.*, 1979; Graf *et al.*, 1984), helps to confirm our hypothesis about the role of iron in the observed peroxidation. Desferroxamine can also form weaker complexes with other metals, such as copper, which could theoretically participate in peroxidation. Compared with iron, however, the relative amounts of these other metals are very small, so that it is not

likely that they contribute appreciably to the effects observed.

The experiments with ADP were performed to ascertain whether chelation by this normal cellular constituent would augment catalytic activity, as has been described in other systems involving iron (Svingen & Aust, 1980; Floyd, 1983; Graf *et al.*, 1984). Whereas significant augmentation was noted with amosite and Canadian chrysotile, the presence of ADP resulted in the inhibition of peroxidation due to crocidolite. The discordant response observed here is consonant with our previous suggestion of differences in the co-ordination state of iron in the different varieties of asbestos (Weitzman & Graceffa, 1984). That some chelators (such as ADP) enhance the catalytic activity of iron is thought to be due to the presence of at least one co-ordination site that is open or occupied by a readily dissociable water ligand (Graf *et al.*, 1984). According to this theory, other iron chelates, such as those with desferroxamine, lack co-ordinated water and hence are inactive catalytically. The effects of chelation are very complex, however, and changes in metal solubility and redox potential also may be important factors (Butler & Halliwell, 1982; Flitter *et al.*, 1983).

The data presented here suggest that lipid peroxidation might be an important mechanism by which asbestos produces tissue damage, and that it might be possible to inhibit this toxic process by treatment of asbestos with chelating agents in the desferroxamine family.

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