Fibre-induced lipid peroxidation leads to DNA adduct formation in Salmonella typhimurium TA104 and rat lung fibroblasts

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Certain end-products of lipid peroxidation bind to DNA forming a fluorescent chromophore. Incubation of both Salmonella typhimurium TA104 and a rat lung fibroblast cell line, RFL-6, with various types of mineral fibre resulted in a time- and dose-dependent increase in DNA fluorescence. The increase in DNA fluorescence was shown to be directly related to the amount of iron that could be mobilized from the fibre surface using in vitro studies in the absence of cells or bacteria. Crocidolite and man-made vitreous fibre-21 (MMVF-21) mobilized significant quantities of iron and were significantly more active than chrysotile and refractory ceramic fibre-1 (RCF-1). Fibre-induced malondialdehyde-DNA adduct formation, the fluorescent product, was increased by incubating cells with buthionine sulfoximine and ameliorated by co-treatment with N-acetylcysteine, indicating a protective role for glutathione. Similarly, vitamin E was also shown to inhibit DNA adduct formation. These results suggest that mineral fibre-induced lipid peroxidation produces genotoxic products which can diffuse into the nucleus and interact with cellular DNA. In conclusion, fibre-induced lipid peroxidation may be a possible mechanism in the genotoxic action of fibrous materials.

Introduction

Exposure to asbestos has been causally linked to the development of several pathogenic pulmonary conditions such as fibrosis, bronchogenic carcinoma and pleural and peritoneal mesothelioma (1,2). Studies in vitro and in animal models have elucidated several important factors in the pathogenesis of asbestos-related diseases, such as fibre size and shape (3,4), surface charge (5) and durability in the lung (6), although the molecular mechanisms are still unclear. Many studies have indicated a role for the iron associated with asbestos, which can constitute up to 36% by weight (7-9). Iron, which has also been implicated as a causative agent in other human cancers (10), is probably active by its ability to catalyze the production of reactive free radical oxygen species such as the superoxide anion and hydroxyl radical (7). These species can react with DNA causing DNA strand breaks (8,11,12) and base modifications (9,13) as well as inducing cellular oxidative stress in which the antioxidant defences are depleted and the normal gene expression is perturbed (14,15). These effects in combination result in altered growth characteristics of the affected tissue and may represent the initiation phase of asbestos-induced carcinogenesis.

Fibres resident in the lung are phagocytosed by fibroblasts, epithelial and mesothelial cells and alveolar macrophages as part of the normal lung response (16,17). During phagocytosis the radical producing surface of the fibre will come into intimate contact with the cellular membrane. Oxygen radicals can initiate lipid peroxidation, a destructive process which results in a chain radical reaction involving lipid, alkoxy and hydroperoxy radicals in the propagation phase and a wide spectrum of aldehydic, ketonic and hydroxy termination products. There is now considerable evidence that the products of lipid peroxidation are involved in carcinogenesis (18). Animals fed diets high in polyunsaturated fats display an increased incidence of tumours and hyperplasias (19), and there is also an apparent relationship between dietary fat intake and incidence of cancer in humans (18).

The mechanisms by which lipid peroxidation may be involved in carcinogenesis are twofold. The lipid derived radicals and their breakdown products can interact directly with DNA. Malondialdehyde (MDA*) and peroxidizing arachidonic acid bind directly to DNA bases forming adducts with characteristic fluorescence spectra (20-22). MDA can form intra- and inter-strand DNA crosslinks and cyclic base adducts (23-25), the latter of which are thought to be the mutagenic lesion reverting frameshift strains of Salmonella typhimurium. MDA has also been shown to be mutagenic in eukaryotic cells causing base adducts and chromosome aberrations (26). Such DNA interactions are a likely source of heriditable mutations in cells. Lipid peroxidation products are also known to affect the cell indirectly by altering cell metabolism and morphology (27,28), binding to cellular proteins (29), inactivating macromolecule synthesis (30) and depleting cellular antioxidants such as glutathione (GSH) (31). In addition it is possible that the presence of lipid peroxidation products modulates the signalling pathways involved in cell proliferation (32).

In this paper we present data suggesting a potential role for fibre-induced lipid peroxidation in DNA adduct formation in Salmonella typhimurium TA104 and a rat lung fibroblasts cell line (RFL-6). The results suggest that the iron associated with fibres catalyzes the lipid peroxidation and that these products bind to DNA. In addition, vitamin E, an inhibitor of lipid peroxidation, and GSH, a cellular antioxidant, have been shown in these studies to have a protective role against this type of damage.

Materials and methods

Chemicals and mineral fibres

Reference samples of Union Internationale Contre le Cancer (UICC) processed crocidolite and chrysotile asbestos fibres were a kind gift from Dr R.C.Brown (MRC Toxicology Unit, Leicester University, Leicester, UK). Man-made Vitreous Fibre-21 (MMVF-21) and Refractory Ceramic Fibre-1 (RCF-1) were obtained from the Thermal Insulation Manufacturers Association; TBARS, thiorbarbituric acid reactive substances; UICC, Union Internationale Contre le Cancer.
Association (TIMA) Fibre Repository (Littleton, CO, USA), α-Tocopherol (vitamin E) (Aldrich), EDTA, hydrogen peroxide, sarcosyl, sodium chloride (BDH), protease K (Boehringer), phenol, phenol/chloroform/isoamyl alcohol (Camlab), Dulbecco's PBS (w/o calcium, magnesium and sodium bicarbonate), Hank's balanced salt solution (w/o calcium and magnesium) (Gibco), nutrient broth no. 2 (Oxoid), DL-buthionine-[S,R]-sulfoximine, calf thymus DNA, lysozyme, N-acetyl cysteine, RNase A (type XII, bovine pancreas), 1,1,3,3-tetraethoxypropane, and tris-(hydroxymethyl)methylamine hydrochloride (Tris-HCl) (Sigma) were used without further purification.

Preparation of malondialdehyde
MDA was prepared from 1,1,3,3-tetraethoxypropane by acidic hydrolysis as described previously (20).

Cell culture of RFL-6 cells
RFL-6 cells (obtained from the European Collection of Animal Cell Cultures, Porton Down, Wiltshire, UK) were grown in Hams F-12 containing L-glutamine (Gibco) supplemented with penicillin (50 U/ml), streptomycin (100 μg/ml), non-essential amino acids (1%; Gibco) and fetal bovine serum (10%; Gibco). Cells were grown to confluence and 24 h prior to addition of mineral dusts, the growth medium was replaced with medium containing 2% serum.

Treatment of isolated DNA, S.typhimurium TA104 and RFL-6 cells with mineral fibres
Isolated DNA in phosphate buffered saline (PBS) (1 mg/ml; 500 μl) was incubated with MDA (10% v/v in PBS; 500 μl) for up to 72 h at 37°C. Reaction mixtures were in a final volume of 2 ml. The experiments were repeated in PBS solutions adjusted to pH 7.5 and 4.75. The DNA was precipitated overnight by addition of 100% ethanol, 33% trichloroacetic acid and 0.3 M sodium acetate (2.5:0.185:0.1 v/v/v) and stored at −20°C.

S.typhimurium TA104 (obtained from Professor B.N.Ames, Berkeley, USA) was cultured in nutrient broth at 37°C for 18 h. An aliquot (500 μl) of this culture was transferred to a 25 ml conical flask containing chrysotile (0.4 mg), crocidolite (0.4 or 0.8 mg), MMVF-21 (0.4 or 0.8 mg) or RCF-1 (0.4 mg) in a final volume of 10 ml and incubated for 24 h in a shaking water bath. For 48 h incubations, the nutrient broth was removed by centrifugation after 24 h and replaced with fresh nutrient broth (20 ml). The broth was then split equally into two flasks and half the concentration of each fibre was added to each flask ensuring an equivalent concentration to the 24 h incubations. To enhance the sensitivity of the assay, H₂O₂ (0.5 mM) was added to the incubations. Additional incubations were carried out with crocidolite (0.4 mg) or MMVF-21 (0.4 mg) in the presence and absence of buthionine sulfoximine (BSO; 1 mM), an inhibitor of γ-glutamyl cysteine synthetase (33), N-acetylcysteine (NAC; 5 mM) or vitamin E (0.5 and 2 mM). The viability of the bacteria at the end of the incubation period was assessed by taking a 100 μl aliquot of the broth and performing a bacterial incorporation assay as described previously (9). The presence of a dense background lawn and revertant colonies after 48 h incubation was considered a marker of bacterial viability.

RFL-6 cells were incubated with mineral fibres for 24 h at 37°C. The fibres were suspended in Hank's Balanced Salt Solution and added directly to the medium at final concentrations of 2 and 5 μg/cm² per dish.

Measurement of GSH levels in S.typhimurium TA104
GSH levels in S.typhimurium TA104 were measured according to the procedure described by Hissin and Hilf (34).

Measurement of lipid peroxidation
Lipid peroxidation was measured using the thiobarbituric acid assay as described previously (8).

Fig. 1. Formation of fluorescent adducts in isolated calf thymus DNA treated in vitro with 10% malondialdehyde at pH 4.75 and 7.5. The results are the mean SD of three separate experiments (n = 6).

Fig. 2. Time- and dose-dependent formation of fluorescent DNA adducts in Salmonella typhimurium TA104 incubated with crocidolite (A) and MMVF-21 (B). The results are the means of four independent determinations. All SD are <2% of the mean value.

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Isolation of bacterial and RFL-6 cell DNA

*S. typhimurium* TA104 cell pellets were resuspended in STE buffer (0.1 M NaCl, 25 mM Tris–HCl containing 1 mM EDTA, pH 7.8; 100 µl) and GTE buffer (50 mM glucose, 25 mM Tris–HCl containing 10 mM EDTA, pH 8.0, with lysozyme [1 mg/ml] added; 1 ml). The cells were incubated at 37°C for 2 h. RFL-6 cell pellets were resuspended in TE buffer (10 mM Tris–HCl containing 1 mM EDTA, pH 8.0; 100 µl) and proteinase K buffer (proteinase K: 100 µg/ml, 5 mM EDTA and 0.5% sarcosyl, pH 8.0) and incubated at 50°C for 2 h. In both cases, DNA was purified by phenol extraction as described previously (35).

**Fluorometric analysis of DNA**

The DNA was quantified by measuring A_{260} (1 absorbance unit = 50 µg DNA) and the purity was monitored using the A_{260}/A_{230} and A_{260}/A_{280} ratios which in the absence of contamination should be ~0.4 and 1.8, respectively. MDA-adducts were measured as described previously (20). Following precipitation, the DNA was dissolved in 'ultra high quality water' (UHQ) (2 ml); obtained from a purification filtering unit purchased from Elga Ltd., High Wycombe, UK and the DNA fluorescence was measured using a Perkin Elmer luminescence spectrometer LS 50B with excitation and emission wavelengths of 390 nm and 460 nm, respectively.

**Iron mobilisation from fibres**

Ferrozine (3.8 ml; 2 mM in phosphate buffer, pH 7.5) was added to 200 µl of fibre suspension (5 mg fibre/ml UHQ water) and the supernatant was removed and analysed spectrophotometrically. The fibres were resuspended in desferrioxamine (4 ml; 2 mM in phosphate buffer, pH 7.5) and the process repeated. The total iron mobilized was recorded as the sum of the ferrozine and desferrioxamine determinations. Standard absorbance curves were obtained using FeCl₃, desferrioxamine and Fe₂(SO₄)₃/ferrozine. The extinction coefficients for desferrioxamine and ferrozine were taken as 2.54 mM⁻¹ and 27.9 mM⁻¹ at 430 nm and 562 nm, respectively.

**Results**

**Fluorescence analysis of DNA**

The reaction of MDA with isolated DNA increased with incubation time and the formation of MDA adducts was pH dependent where more adducts were produced at pH 4.75 than at pH 7.5 (Figure 1). Incubation of *S. typhimurium* TA104 with crocidolite asbestos or MMVF-21 produced a significant time-dependent increase in fluorescence of a similar magnitude (Figure 2 and Figure 3; P < 0.005). Pre-treatment of fibres with desferrioxamine and ferrozine prior to incubation with TA104 significantly reduced the formation of the fluorescent adduct (Figure 4; P < 0.05). Incubation of TA104 with chrysotile and RCF-1 resulted in small but statistically significant increase over background fluorescence (Figure 3; P < 0.02). In addition, H₂O₂ caused a small increase over background DNA fluorescence in the absence of fibres at both 24 h and 48 h. The occurrence of lipid peroxidation was verified by measuring the formation of thiobarbituric acid reactive substances (TBARS). In these experiments, dose-dependent increases in TBARS were seen in *S. typhimurium* at 24 h (Table I) and at 48 h (Table II) and in RFL-6 cells at 24 h with both crocidolite and MMVF-21 (Table III).

**Iron mobilisation from fibres**

Crocidolite and MMVF-21 mobilized significantly more iron than the other fibres (Table IV). The ratio of Fe^{II} to Fe^{III} was approximately 1:1 in all fibres examined except MMVF-21. In MMVF-21, over 90% of the mobilized iron was removed by ferrozine, indicating it to be in the Fe^{III} form. In addition it was observed that MMVF-21 released its iron much more rapidly than the other fibres (unpublished observation).

**Measurement of GSH levels in S. typhimurium TA104**

Following treatment of *S. typhimurium* TA104 with BSO and NAC, GSH levels were significantly decreased and increased, respectively (Table V).

**Modulation of fibre activity**

To investigate whether GSH has a protective role against fibre-induced lipid peroxidation, *S. typhimurium* TA104 was treated with crocidolite or MMVF-21 in the presence of BSO (1 mM), an inhibitor of γ-glutamyl cysteine synthetase (37). The DNA fluorescence in these bacteria following fibre treatment was significantly greater than in bacteria without BSO pretreatment (Figure 4; P < 0.05). Co-treatment with NAC, a GSH precursor, produced fluorescent adduct levels similar to those in bacteria without BSO and NAC treatment. Vitamin E (α-tocopherol), an inhibitor of lipid peroxidation, protected TA104 against fluorescent DNA adduct formation (Figure 5; P < 0.05).

![Fig. 3. Time-dependent formation of fluorescent DNA adducts in *Salmonella typhimurium* TA104 incubated with crocidolite (0.4 mg), MMVF-21 (0.4 mg), chrysotile (0.4 mg) and RCF-1 (0.4 mg) in the presence of H₂O₂ (0.5 mM). The results are the means of four independent determinations. All SD are <10% of the mean value.](https://academic.oup.com/carcin/article-abstract/17/3/413/312863)
Table I. Lipid peroxidation as measured by the formation of TBARS in Salmonella typhimurium TA104 after incubation with crocidolite or MMVF-21 for 24 h in the presence or absence of BSO (1 mM), NAC (5 mM) or vitamin E

<table>
<thead>
<tr>
<th>Fibre</th>
<th>Treatment</th>
<th>None</th>
<th>BSO</th>
<th>BSO + NAC</th>
<th>BSO + vitamin E (0.5 mM)</th>
<th>BSO + vitamin E (2 mM)</th>
<th>H₂O₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>0.18±0.06*</td>
<td>0.72±0.02</td>
<td>0.60±0.30</td>
<td>0.55±0.13</td>
<td>0.44±0.21</td>
<td>4.64±2.74</td>
</tr>
<tr>
<td>Crocidolite (0.4 mg)</td>
<td></td>
<td>1.13±0.03</td>
<td>1.14±0.17</td>
<td>0.47±0.04</td>
<td>0.67±0.17</td>
<td>0.58±0.21</td>
<td>7.7±2.6</td>
</tr>
<tr>
<td>Crocidolite (0.8 mg)</td>
<td></td>
<td>1.39±0.27</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MMVF-21 (0.4 mg)</td>
<td></td>
<td>0.95±0.06</td>
<td>0.76±0.60</td>
<td>1.55±1.86</td>
<td>-</td>
<td>-</td>
<td>8.2±3.1</td>
</tr>
<tr>
<td>MMVF-21 (0.8 mg)</td>
<td></td>
<td>1.69±0.86</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*The data represent nmol TBARS/mg protein and are the means ± SD of four separate determinations.

Table II. Lipid peroxidation as measured by the formation of TBARS in Salmonella typhimurium TA104 after incubation with crocidolite or MMVF-21 for 48 h in the presence or absence of BSO (1 mM), NAC (5 mM) and vitamin E

<table>
<thead>
<tr>
<th>Fibre</th>
<th>Treatment</th>
<th>None</th>
<th>BSO</th>
<th>BSO + NAC</th>
<th>BSO + vitamin E (0.5 mM)</th>
<th>BSO + vitamin E (2 mM)</th>
<th>H₂O₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>0.50±0.21</td>
<td>1.19±0.67</td>
<td>0.80±0.23</td>
<td>0.67±0.33</td>
<td>0.21±0.04</td>
<td>5.50±1.98</td>
</tr>
<tr>
<td>Crocidolite (0.4 mg)</td>
<td></td>
<td>2.17±1.02</td>
<td>3.20±1.17</td>
<td>2.50±0.42</td>
<td>1.90±0.27</td>
<td>1.34±0.31</td>
<td>10.77±1.9</td>
</tr>
<tr>
<td>Crocidolite (0.8 mg)</td>
<td></td>
<td>6.16±0.72</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MMVF-21 (0.4 mg)</td>
<td></td>
<td>2.26±1.36</td>
<td>2.90±0.32</td>
<td>2.10±0.51</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MMVF-21 (0.8 mg)</td>
<td></td>
<td>7.26±1.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*The data represent nmol TBARS/mg protein and are the means ± SD of four separate determinations.

Table III. Lipid peroxidation as measured by the formation of TBARS in RFL-6 cells following incubation with crocidolite or MMVF-21 in the presence or absence of BSO (1 mM), NAC (5 mM) and vitamin E

<table>
<thead>
<tr>
<th>Fibre</th>
<th>Treatment</th>
<th>None</th>
<th>BSO</th>
<th>BSO + NAC</th>
<th>BSO + vitamin E (0.1 mM)</th>
<th>BSO + vitamin E (0.5 mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>218±105*</td>
<td>286±85</td>
<td>207±82</td>
<td>ND</td>
<td>285±66</td>
</tr>
<tr>
<td>Crocidolite (2 μg/cm²)</td>
<td></td>
<td>307±12</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Crocidolite (5 μg/cm²)</td>
<td></td>
<td>502±18</td>
<td>657±77</td>
<td>140±12</td>
<td>277±51</td>
<td>215±72</td>
</tr>
<tr>
<td>MMVF-21 (2 μg/cm²)</td>
<td></td>
<td>344±214</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>MMVF-21 (5 μg/cm²)</td>
<td></td>
<td>485±81</td>
<td>671±62</td>
<td>116±32</td>
<td>350±28</td>
<td>297±24</td>
</tr>
</tbody>
</table>

*All values are pmol TBARS/mg protein and are the mean ± SD of four separate experiments.
*ND, not determined.

Table IV. Iron mobilisation from crocidolite, chrysotile, MMVF-21 and RCF-1 as determined spectrophotometrically by desferrioxamine and ferrozine iron chelation

<table>
<thead>
<tr>
<th>Fibre type</th>
<th>Fe²⁺ (nmol/mg)</th>
<th>Fe³⁺ (nmol/mg)</th>
<th>Total iron (nmol/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crocidolite</td>
<td>69*</td>
<td>80</td>
<td>149</td>
</tr>
<tr>
<td>Chrysotile</td>
<td>33</td>
<td>36</td>
<td>69</td>
</tr>
<tr>
<td>MMVF-21</td>
<td>103</td>
<td>18</td>
<td>121</td>
</tr>
<tr>
<td>RCF-1</td>
<td>5</td>
<td>5</td>
<td>10</td>
</tr>
</tbody>
</table>

*The results are the means of two determinations.

Discussion

The present study has demonstrated that incubation of bacteria and mammalian cells with several fibre forms results in a dose- and time-dependent increase in DNA fluorescence. In these experiments, H₂O₂ was added to the assay with bacteria to enhance the sensitivity of the assay. Previous investigations have shown that the activity of fibres is enhanced in the presence of H₂O₂ and organic peroxides (9,37,38). The same fluorescence is obtained from the in vitro reaction between MDA and isolated DNA. Similar fluorescence spectra are also obtained from the reaction between DNA and other products of lipid peroxidation such as peroxidizing arachidonic acid. These DNA adducts have been shown in vitro to alter the physico-chemical nature of DNA (20-22) and may be a source of DNA damage.
Table V. Glutathione content of Salmonella typhimurium TA104 following 18 h culture in nutrient broth or nutrient broth containing NAC (5 mM), BSO (1 mM) or NAC (5 mM) and BSO (1 mM)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Glutathione concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>94 ± 32*</td>
</tr>
<tr>
<td>BSO (1 mM)</td>
<td>38 ± 22</td>
</tr>
<tr>
<td>BSO (1 mM) + NAC (5 mM)</td>
<td>154 ± 48</td>
</tr>
<tr>
<td>NAC (5 mM)</td>
<td>164 ± 9</td>
</tr>
</tbody>
</table>

*Values are nmol GSH/mg protein and are the means ± SD of four independent determinations.

![Graph](https://example.com/graph.png)

Fig. 5. The effect of vitamin E (0.5 and 2 mM) on crocidolite-induced formation of fluorescent DNA adducts in Salmonella typhimurium TA104. The results are the means of four independent determinations. All SD are <20% of the mean value.

![Graph](https://example.com/graph2.png)

Fig. 6. Formation of fluorescent DNA adducts in RFL-6 cells treated in vitro as follows: (1) control; (2) BSO (1 mM); (3) BSO (1 mM) + NAC (5 mM); (4) crocidolite (2 μg/cm²) + BSO (1 mM); (5) crocidolite (2 μg/cm²) + BSO (1 mM) + NAC (5 mM); (6) MMVF-21 (2 μg/cm²) + BSO (1 mM); (7) MMVF-21 (2 μg/cm²) + BSO (1 mM) + NAC (5 mM); (8) crocidolite (5 μg/cm²) + BSO (1 mM); (9) crocidolite (5 μg/cm²) + BSO (1 mM) + NAC (5 mM); (10) MMVF-21 (5 μg/cm²) + BSO (1 mM); (11) MMVF-21 (5 μg/cm²) + BSO (1 mM) + NAC (5 mM); (12) crocidolite (5 μg/cm²) + BSO (1 mM) + vitamin E (0.1 mM); (13) crocidolite (5 μg/cm²) + BSO (1 mM) + vitamin E (0.5 mM); (14) MMVF-21 (5 μg/cm²) + BSO (1 mM) + vitamin E (0.1 mM); (15) MMVF-21 (5 μg/cm²) + BSO (1 mM) + vitamin E (0.5 mM). The results are the means ± SD of four independent determinations.

Fig. 7. Formation of fluorescent DNA adducts in RFL-6 cells treated in vitro as follows: (1) control; (2) BSO (1 mM); (3) BSO (1 mM) + NAC (5 mM); (4) crocidolite (2 μg/cm²) + BSO (1 mM); (5) crocidolite (2 μg/cm²) + BSO (1 mM) + NAC (5 mM); (6) MMVF-21 (2 μg/cm²) + BSO (1 mM); (7) MMVF-21 (2 μg/cm²) + BSO (1 mM) + NAC (5 mM); (8) crocidolite (5 μg/cm²) + BSO (1 mM); (9) crocidolite (5 μg/cm²) + BSO (1 mM) + NAC (5 mM); (10) MMVF-21 (5 μg/cm²) + BSO (1 mM); (11) MMVF-21 (5 μg/cm²) + BSO (1 mM) + NAC (5 mM); (12) crocidolite (5 μg/cm²) + BSO (1 mM) + vitamin E (0.1 mM); (13) crocidolite (5 μg/cm²) + BSO (1 mM) + vitamin E (0.5 mM); (14) MMVF-21 (5 μg/cm²) + BSO (1 mM) + vitamin E (0.1 mM); (15) MMVF-21 (5 μg/cm²) + BSO (1 mM) + vitamin E (0.5 mM). The results are the means ± SD of four independent determinations.

Fibre-induced DNA adduct formation

Fibre-induced DNA adduct formation

of mutation in vivo. MDA has a pKₐ of 4.46 and therefore at physiological pH exists as the enolate ion which is much less reactive towards nucleophiles than the β-hydroxynicolein form that exists at lower pH (23). Therefore under physiological conditions the extent of MDA-DNA binding is likely to be small but possibly still biologically significant. Indeed, cyclic MDA-DNA adducts are thought to be responsible for the mutagenicity of MDA in frameshift strains of S. typhimurium (23, 24).

Many previous studies have indicated a role for fibre-bound iron in the activity of fibres (8, 9, 11, 12). The present study has confirmed these observations since the ability of a fibre type to induce lipid peroxidation is directly related to its potential maximal mobilisable iron content from in vitro experiments in the absence of bacteria and RFL-6 cells. In addition, pre-treatment of fibres with iron chelators significantly reduces fibre-induced DNA adduct formation.

Gulumian and co-workers have shown that fibres induce lipid peroxidation and that the spectrum of end-products formed varies between fibres in both quantity and type (39). Since some of these products are highly biologically active it is possible that the spectrum of lipid peroxidation products induced by a particular fibre type may be related to its fibrogenic and/or carcinogenic nature (40, 41). Products of lipid peroxidation can act as genotoxic agents by directly interacting with DNA and also via non-genotoxic mechanisms. For example, MDA acts as an initiator in mouse skin (42) and also alters the growth characteristics and synthesis of macromolecules in mammalian cells (27, 28). Lipid peroxidation has also been shown to be involved in the modulation of cell proliferation (32). The presence of lipid hydroperoxides induces ornithine decarboxylase (ODC), a gene product intimately involved with cell proliferation (43). ODC is also expressed in hamster tracheal epithelial cells after exposure to asbestos although whether this effect is a direct result of oxygen radicals or lipid peroxidation products was not investigated (44). GSH is known to play a protective role against oxidative stress and lipid peroxidation (45). Studies in isolated DNA have shown a protective role for GSH in free radical damage produced by the iron on the fibre surface using 8-hydroxydeoxyguanosine as a biomarker of free radical DNA damage (P.J. Howden and S.P. Faux, unpublished observations). Previous studies have shown that following treatment of rat pleural mesothelial cells with BSO or NAC levels of GSH are altered within these cells, where BSO and NAC decreased and increased GSH pools, respectively (15). In this investigation, we have shown that a similar effect is observed in S. typhimurium TA104.
following BSO and NAC incubation. The present study has demonstrated a protective role for both GSH and vitamin E against this type of damage. These agents are probably behaving as free radical scavengers. Persistent exposure to fibres will deplete the cells of endogenous levels of these agents and this may have important consequences. Intracellular GSH levels have recently been shown to modulate c-fos and c-jun expression in cells following exposure to asbestos (15) and the presence of vitamin E has been shown to negate the perturbation of cell proliferation caused by lipid peroxidation (32). There is now increasing evidence that the activation of these genes by inhaled fibres leads to chronic cell proliferation and it is possible that lipid peroxidation represents one mechanism in the modulation of this process.

In summary, the data presented here suggest that fibres induce lipid peroxidation via an iron-dependent mechanism, and the products formed can interact with DNA to form adducts. GSH and vitamin E have protective roles probably against this type of damage. These agents are probably behaving

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References


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