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Asbestos Affects the *in Vitro* Uptake and Detoxification of Aromatic Compounds

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A number of mineral dusts were tested for their ability to catalyze the transformation of benzo(a)pyrene from the microcrystalline state into lipid solution. The findings of Lakowicz and his coworkers, that fibrous dusts were more active than nonfibrous dusts, were confirmed. Macromolecular binding metabolites of BaP were formed in A549 cells to a similar extent whether the BaP was added in solution or adsorbed to fibers; however, the level of water-soluble metabolites was lower in cultures treated with adsorbed hydrocarbon. It was found that asbestos can also inhibit the accumulation of 1-naphthyl glucuronide in cultures treated with 1-naphthol. The significance of this in asbestos pathogenesis is briefly discussed.

Introduction

It is our opinion that despite extensive experimentation there has been no unequivocal demonstration of the short-term genotoxicity of fibrous dusts; thus the carcinogenic activities of these materials remain largely unexplained. There are several possible explanations for this, but perhaps the most likely are: (1) fibrous dusts could be so weakly carcinogenic that they only exert their *in vivo* effects by virtue of their long residence times in damaged tissue or (2) they act by enhancing the activities of other agents even when apparently acting alone.

In this paper we address ourselves primarily to this second possibility.

There are several possible mechanisms whereby fibrous dusts could act as "carcinogenic enhancers," although the one which has received the most attention is that fibers could act as "carriers" mediating the transfer of carcinogens into tissue by holding them *in situ* for longer periods than would be the case for the carcinogen alone. Lakowicz and his coworkers (*1*, *2*) have demonstrated that fibers can catalyze the transfer of hydrocarbons into solution in lipid membranes. The joint effects of fibers and chemical carcinogens have recently been reviewed (*3*). We have been investigating the effect of various dusts on the metabolism of benzo(a)pyrene in several types of cells *in vitro* in an attempt to study phenomena occurring after any enhanced uptake.

Materials and Methods

Cells

Human alveolar epithelial type II tumor cells (A549) (*4*) were grown in Dulbecco's MEM (DMEM) containing 10% heat inactivated fetal calf serum and antibiotics. Primary rat embryo fibroblasts were obtained by digesting minced 20-day rat embryos with 0.5% trypsin in Hank's balanced salt solution at pH 6.9 and room temperature. The digest was filtered through sterile gauze, and the cells were washed, counted and plated out at 10^6 cells in each 75 cm^2 tissue culture flask containing similar medium to that used for the A549 cells above. P388D1 cells (*5*) were cultured in DMEM supplemented with 10% heat-inactivated newborn calf serum.

Rat liver microsomes were prepared by homogenizing liver in three times its volume of Tris buffer (0.05 *M*, pH 7.4) containing 0.154 *M* KCl. The homogenate was filtered through surgical gauze then centrifuged at 16000*g* and the supernatant decanted and respun at 105,000*g* for 1.5 hr. The pellets were resuspended by sonication, and the suspensions stored frozen at − 20°C.

Chemicals

[^{3}H]-Benzo(a)pyrene (^{3}H-BaP) and [^{14}C]-1-naphthol were obtained from Amersham International P.L.C.;

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BaP, 1-naphthol, 1-naphthyl glucuronide and 1-naphthyl sulfate were obtained from the Sigma Chemical Co., Poole, Dorset.

Both tritiated and "cold" BaP were purified before use by dissolving in petroleum spirit (60-80°C boiling range) and chromatography through a silica "Sep Pak" (Water Associates, Milford, MA). The purified BaP was dried down in a stream of nitrogen and taken up in acetone before use. BaP was adsorbed to dust surfaces by adding the acetone solution to an ethanolic suspension of dust and drying in a stream of nitrogen at 80°C.

All cultures were treated with asbestos suspension and/or aromatic compound on the day that confluence was reached. Primary fibroblasts were used before passage 5. Both cell lines were of indeterminate passage number.

Dusts

The UICC asbestos samples (*6*) were used; milled amosite and crocidolite were prepared as described previously (*7*). The pulverized fuel ash sample was obtained from Didcot Power Station, Buckinghamshire, England.

Analyses

Fluorescence measurements were made on a Perkin-Elmer spectrofluorimeter model 3000.

HPLC separations were carried out using two Altex 110A pumps connected to an Altex microprocessor controller. An LDC Spectromonitor III UV detector and a Gilson fraction collector were used. Columns were obtained from Jones Chromatography, Llanbradach, Wales.

Radioactivity measurements were made on an Intertechnique SL 4000 liquid scintillation counter with on-line DPM corrections made by the external standard method.

Results and Discussion

The transfer of BaP from the microcrystalline condition to lipid solution was studied, by using methods based on those described previously (*2*) it was found that UICC amosite was far more efficient in promoting this transfer than the essentially nonfibrous milled derivative. Pulverized fuel ash did not accelerate the transfer (Fig. 1).

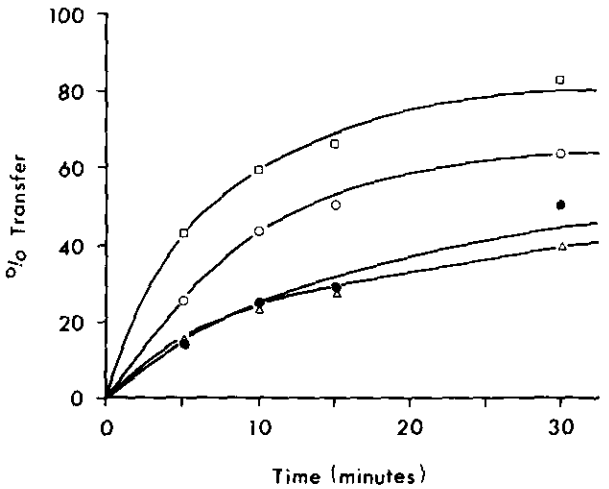

FIGURE 1. Rat liver microsomes were resuspended to a final protein concentration of 0.006 mg protein/mL and incubated in a shaking water bath at 37°C: (□) UICC amosite; (○) milled amosite; (•) BAP microcrystals; (△) pulverized fuel ash. Dusts were added to the microsome suspension to a final concentration of 0.167 mg/mL and the fluorescence spectrum of samples read at various times. The transfer was calculated by heating the residual suspension to 55°C for 1 hr and performing the appropriate calculations as described by Lakowicz and Hylden (*1*).

It was therefore decided to study the effect on metabolism (in cultured cells) of adding the hydrocarbons in the adsorbed state. This was felt particularly relevant in view of the report that, although hydrocarbon transfer to microsomes was increased by fibers, the formation of metabolites was actually inhibited (*8*). Cultures of A549 cells were set up with BaP, under the conditions described in Table 1. The various classes of metabolite were measured as described by Grover et al. (*9*). As can be seen, the addition of BaP in the adsorbed state resulted in a reduction in the level of water-soluble metabolites in the medium but did not lower the level of covalently bound hydrocarbon; indeed, this increased in one series of cultures. Thus, the fall in conjugated metabolites appears to be a selective toxic effect rather than the result of overall cell damage. The wide variation in the metabolite levels between experiments seems to be unavoidable; attempts to induce hydrocarbon metabolism in these cells to a maximum and reproducible level have failed.

In the light of these results it was decided to study the ability of A549 cells to conjugate 1-naphthol, thus removing the oxidation steps from consideration. The formation of 1-naphthyl glucuronide and 1-naphthyl sulfate was studied by using ion pair HPLC as described by Karakaya and Carter (*10*). From Figure 2 it can be seen that conjugation of this substrate in these cells is mainly to the glucuronide.

The effect of crocidolite asbestos on the conjugation reaction is shown in Figure 3. It can be seen that there is significant inhibition with the UICC crocidolite but much less with the milled derivative, although neither dust had a significant effect at 50 μg/mL.

Table 1. Cell treatment with hydrocarbons and metabolite level.[a]

Group	Treatment	Medium: Water-soluble metabolites, nmole/mg protein	Medium: Ether-soluble metabolites, nmole/mg protein	Cells: Protein-bound metabolites, pmole/mg protein	Cells: DNA-bound metabolites, pmole/mg protein	Cells: RNA-bound metabolites, pmole/mg protein
1	8 μM BaP in solution	2.93 ± 0.79	1.35 ± 0.23	28.6 ± 1.65	0.48 ± 0.11	1.14 ± 0.11
	8 μM BaP 100 μg crocidolite/mL	2.12 ± 0.17	1.52 ± 0.04	26.9 ± 3.10	0.46 ± 0.02	1.21 ± 0.26
2	15 μM BaP solution	10.15 ± 1.05	0.63 ± 0.03	12.5 ± 0.9	0.09 ± 0.003	0.28 ± 0.03
	15 μM BaP adsorbed to 100 μg crocidolite/mL	4.55 ± 0.4	0.66 ± 0.08	18.9 ± 4.6	0.155 ± 0.02	0.40 ± 0.04
3	15μM BaP solution	4.69 ± 1.17	1.68 ± 0.08	21.1 ± 2.8	0.89 ± 0.02	0.11 ± 0.02
	15 μM BaP adsorbed to 200 μg crocidolite/mL	2.39 ± 0.17	1.20 ± 0.12	20.6 ± 2.4	0.84 ± 0.08	0.14 ± 0.02

[a]Cultures of A549 cells were treated as described in the text under the conditions in column 2. The metabolism was measured by using the methods described by Grover et al. (9). The figures in the table are the means of at least two replicates together with standard errors.

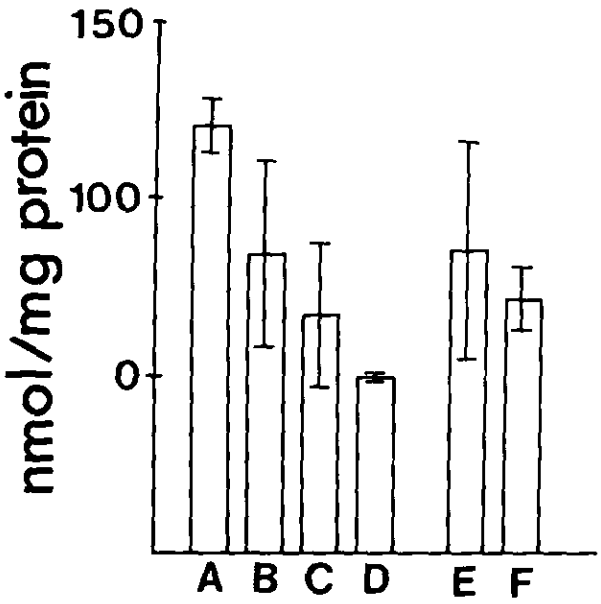

FIGURE 2. Separation of 1-naphthol and its conjugates on HPLC: (top) trace obtained from medium immediately after adding [^{14}C]1-naphthol; (bottom) trace for similar medium after 24 hr in contact with A549 cells. Positions 1, 2 and 3 mark the retention times of authentic 1-naphthyl glucuronide, 1-naphthyl sulfate and 1-naphthol, respectively.

Further studies have shown a similar inhibition in primary rat fibroblasts and a much more complex situation in cultures of the macrophage like P388D1 cell line (Table 2). In these latter cells there is an early stimulation which disappears later, possibly as a result of the combined effect of increased uptake and later inhibition.

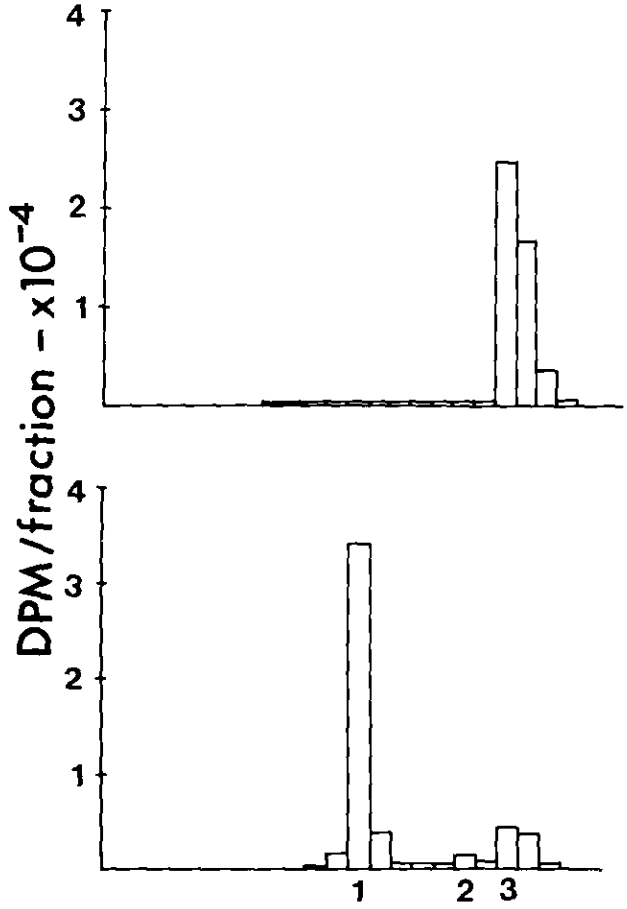

FIGURE 3. Formation of 1-naphthyl glucuronide in the presence of crocidolite asbestos. The mean or standard error is shown for each group. (A) control; (B) crocidolite, 50 μg/mL; (C) crocidolite, 100 μg/mL; (D) crocidolite, 200 μg/mL; (E) milled crocidolite, 50 μg/mL; (F) milled crocidolite, 200 μg/mL.

Table 2. 1-Naphthyl glucuronide formation.

Cell type	Treatment	Time, hr	1-Naphthyl glucuronide formed, nmole/mg protein
Rat embryo fibroblasts	1-Naphthol, 25 μM	8	24.6
		24	69.3
		72	55.7
	1-Naphthol, 25μM + crocidolite, 100μg/mL	8	24.7
		24	46.5
		72	35.1
P388D1	1-Naphthol, 500 μM	6	13.9 ± 4.5
		18	33.8 ± 3.1
	1-Naphthol 500 μM + crocidolite 100 μg/mL	6	20.0 ± 8.1
		18	49.4 ± 5.0
	1-Naphthol, 500 μM + crocidolite, 200 μg/mL	6	19.6 ± 4
		18	25.4 ± 4.4
	1-Naphthol, 500 μM + chrysotile, 100 μg/mL	6	28.6 ± 4.6
		18	38.2 ± 7.0
	1-Naphthol, 500 μM + chrysotile, 200 μg/mL	6	25.4 ± 1.4
		18	36.1 ± 3.5

We would suggest that asbestos can mediate two phenomena: (1) the increased uptake of xenobiotics and (2) the inhibition of glucuronide accumulation. Under some circumstances these two phenomena can lead to increased DNA binding and biological activity of the hydrocarbon (Table 1) (*11*). The mechanism by which glucuronide accumulation is inhibited may be related to the much-studied release of lysosomal enzymes—especially β-glucuronidase—by dust-treated cells. It is unlikely, however, that the acid hydrolases show profound activity at the extracellular pH. Alternatively, it could involve the uptake and hydrolysis of preformed glucuronide by mechanisms similar to those responsible for increased uptake of the parent aglycone. These phenomena could increase the levels of toxic xenobiotic metabolites in asbestos-damaged tissue and increase the probability of malignant transformation (or other damage) in these areas.

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