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Diminution in Phase I and Phase II Drug Metabolizing Enzymes of Rat Lung by Asbestos: An *In Vitro* Study

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The association with smoking habits of increased risk of development of bronchogenic carcinoma (lung cancer) among populations occupationally exposed to asbestos has been very-well documented in epidemiological studies (Selikoff et al., 1980, Mossman, 1988). Numerous experimental studies support the view that asbestos is a co-carcinogen or tumor promoter and further indicated the synergistic action of these mineral fibres with the carcinogenic constituents of the particulate phase of cigarette smoke such as polynuclear aromatic hydrocarbons (PAHs) and nitrosamines in the induction of lung cancer (Salk and Vosamae 1975, Rosin, 1985). It has been proposed that asbestos varieties causing lung cancer act as carriers of certain carcinogenic PAHs, such as benzo(a)pyrene to cellular targets (Lakowicz et al., 1978; Lakowicz and Bewan, 1979), thereby producing the carcinogenic effects. This proposal has not been accepted, as some of the non-carcinogenic particulate materials also transport these carcinogens (Harvey et al., 1984). Thus, the precise mechanisms by which these mineral fibres potentiate the development of lung cancer is still obscure. One of the possible mechanisms by which asbestos can enhance the development of lung cancer in smokers exposed to asbestos may be related to their effects on phase I and phase II drug metabolizing enzymes, as the tissue levels of these enzymes are crucial for deciding the metabolic fate of the carcinogenic constituents of cigarette smoke and other chemical carcinogens (Minchin and Boyd, 1983). In the present paper, therefore, studies are presented concerning the effect of three varieties of asbestos namely, chrysotile, crocidolite and amosite, and of titanium dioxide, an inert non-asbestos dust, on the enzymes of phase I and phase II drug metabolism in isolated rat lung microsomes and post-microsomal

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fraction. Since 3-methylcholanthrene (3-MC) is known to induce cytochromes P-450 in the IA family and their associated activity, benzo(a)pyrene hydroxylase (Minchin and Boyd, 1983), therefore, the effect of these mineral fibres on cytochrome P-450 and benzo(a)pyrene hydroxylase in lung microsomes isolated from 3-MC-treated rats was studied.

MATERIALS AND METHODS

Three UICC standard reference asbestos samples, chrysotile, crocidolite and amosite,particle size below 30 micron, were obtained as a gift from Dr. J.B. Leinweber, John-Manville Mills, USA. All the chemicals and reagents were either procured from Sigma, USA or Sisco Research Laboratory, India and were of analytical grade. For the induction studies, female albino Wistar rats from the ITRC Colony weighing 150-180 gm were injected with 3-methylcholanthrene (3-MC), dissolved in 0.5 ml of corn oil (20 mg/kg body wt.) intraperitoneally for four consecutive days. The control animals received only vehicle. The animals were housed in an air-conditioned room with the arrangement of 12 hours dark and light cycles. The animals were maintained on commercial pellet diet, supplied by Hindustan Lever Ltd., India and tap water ad libitum. The rat lung microsomal fraction was isolated by the procedure of Johannesen et al. (1977). The microsomes were suspended in isotonic (0.15 M KCl), 0.05 M Tris-HCl buffer, pH 7.4, containing 2% glycerol and 0.1 mM EDTA. The animals were sacrificed by decapitation between 9-10 AM to eliminate diurnal variation(Jori et al., 1971).

The adsorption of cytochrome P-450 and its 3-MC-inducible isozymes on the asbestos fibres was measured in the reaction mixtures containing microsomes and dusts (1 mg fibres/mg protein). Following 1 hour incubation at 37 ^{o} C,the dust was removed by centrifugation (3000 x g, 10 minutes) and the cytochrome P-450 content of the supernatant was determined by the method of Omura and Sato (1964) from the carbon monoxide-difference spectra of dithionite-reduced microsomes, based on an extinction coefficient of 91 $mM^{-1} cm^{-1}$. These values were compared with the controls containing only microsomes and microsomes with similar amount of inert dust, titanium dioxide (TiO_2). The difference between control and dust treatment was taken as the adsorption of cytochrome P-450 proteins. For the heme release studies, the incubation was done for 72 hours at 37^{o} C with occasional shaking and in 105,000 x g supernatant heme was measured as pyridine-hemochromogen (Falk, 1974). Substantial heme release was observed at 72 hours, but not at earlier times. For enzyme activities, microsomes and cytosol were

incubated separately with 500 µg dust/mg protein at 37° C for 1 hour with constant stirring. Dusts were separated out by centrifugation. Benzo(a)pyrene hydroxylase activity in supernatant was assayed by the fluorometric technique, using 3-hydroxy-benzo(a)pyrene as standard (Dehnen et al., 1973). Epoxide hydrolase activity was determined by fluorometric technique according to the method of Dansette et al. (1979), using styrene epoxide as a substrate. Glutathione-S-transferase activity was monitored by the spectrophotometric procedure (Habig et al., 1974), using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate. The selection of the dose 1 mg dust/mg protein for the adsorption studies and 0.5 mg dust/mg protein in enzyme studies is based on our unpublished dose-response studies in which maximum effects were observed at these doses. Protein was estimated colorimetrically by the method of Lowry et al. (1951), using bovine serum albumin as a standard.

Statistical significance was determined using one-tailed student's 't' test and a value of $P < 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

The present study show that asbestos produces significant alterations in some phase I and phase II drug metabolizing enzymes of the lung. The incubation of rat lung microsomes with three different varieties of asbestos used separately at 37° C for 1 hour not only resulted in the adsorption of cytochrome P-450 and its 3-MC-inducible isozymes (Table 1), but also inhibited the activity of cytochrome P-450-dependent monooxygenase (such as benzo(a)pyrene hydroxylase activity) as recorded in Table 2. On prolonged incubation for 72 hours heme was released by chrysotile and crocidolite only from microsomal heme-proteins (Table 1). Taking all these things together, it seems that structural damage to cytochrome P-450 assembly may be responsible for the inhibition in the activity of benzo(a)pyrene hydroxylase. The inhibition in the activity of cytochrome P-450-dependent monooxygenase may affect the clearance of the carcinogenic PAHs, such as benzo(a)pyrene, a ubiquitous environmental pollutant,which require metabolic activation for their mutagenic and carcinogenic action (Gelboin, 1980). Asbestos fibres also inhibit the activities of microsomal epoxide hydrolase and cytosolic glutathione-S-transferase (Figure 1). However, no differences in these parameters could be recorded between the untreated controls and controls incubated under the same experimental protocols with an inert dust, titanium dioxide. Epoxide hydrolase and glutathione-S-transferase respectively convert PAH-epoxides to

Table 1 Adsorption of heme-proteins and release of heme by asbestos fibre from lung microsomes

Reaction mixture components	Cytochrome P-450 adsorption		Release of heme**	
	Control*	3-MC treated*	Control	3-MC treated
Microsomes + Titanium dioxide	0.00	0.00	0.00	0.00
Microsomes + Chrysotile	15 ± 1.0	25.6 ± 1.0	4.84 ± 0.21	10.50±0.82
Microsoems + Crocidolite	10 ± 0.75	19.5 ± 1.4	2.59 ± 0.13	6.84±0.53
Microsomes + Amosite	6 ± 0.44	10.1 ± 0.6	ND	ND

*p mole cytochrome P-450 and its 3-MC-inducible isozymes adsorbed/mg asbestos fibres.
**p mole heme released/mg asbestos fibres.
ND: Not detectable
The microsomes isolated from control and 3MC-treated rats were incubated with asbestos fibres (1 mg dust/mg protein) at 37° C. The adsorption of heme protein was studied after 1 hour while heme release observed only after 72 hours of incubation at 37°C with occasional shaking. Control values, mean ± SE (n=6) of cytochrome P-450 and its 3-MC-inducible isozymes respectively were 72±4.85 and 115±5.92 pmole/mg protein. Data represent the mean ± SE of six experiments.

Table 2 Effects of asbestos fibre on the benzo(a)pyrene hydroxylase activity in lung microsomes isolated from untreated and 3MC-treated rats

Reaction mixture components	Benzo(a)pyrene [B(a)P]hydroxylase (pmole 3-OH B(a)P formed/min/mg protein)	
	Control	3-MC treated
Microsomes	11.60±0.91	49.75±4.50
Microsomes + Titanium dioxide	10.50±0.94	50.42±4.90
Microsomes + Chrysotile	7.42±0.46[b]	18.10±2.31[a]
Microsomes + Crocidolite	7.85±0.52[b]	22.30±2.53[a]
Microsomes + Amosite	8.70±0.82[c]	28.45±3.72[a]

The enzyme activities in microsomes were determined by incuibating with 500 µg dust/mg protein at 37° C for 1 hour with constant stirring. 3000 x g supernatant was used as enzyme source. The values represent the mean of six experiments ± SE. Letters a, b and c represent the statistical significance, when compared to controls, [a]$p < 0.001$; [b]$p < 0.01$; [c]$p < 0.05$

dihydrodiols and to mercapturic acid derivatives (Oesch, 1972; Anders et al., 1988) and thus, provide fast excretion/detoxification of toxic exogenous and endogenous compounds before their toxic manifestations become apparent. The inhibition in the activities of these enzymes, therefore, may impair the metabolic disposition of the ultimate carcinogens which require hydrolysis and conjugation prior to their excretion from the lung. The accumulated reactive metabolites in the lungs may then generate a variety of toxic effects, including mutations and cancer (Gelboin, 1980). Furthermore, the release of heme from microsomal heme-proteins by asbestos suggest that these species, by generating reactive oxygen species via Haber-Weiss, Fenton-like reaction, may play a role in the promotion of the multistage process of chemical carcinogenesis (Haber and Weiss, 1934, Yisun, 1990). The mechanisms by which asbestos deactivates cytochrome P-450 and catalyzes its decomposition, releasing heme, are not clear. However, it is possible that an asbestos-

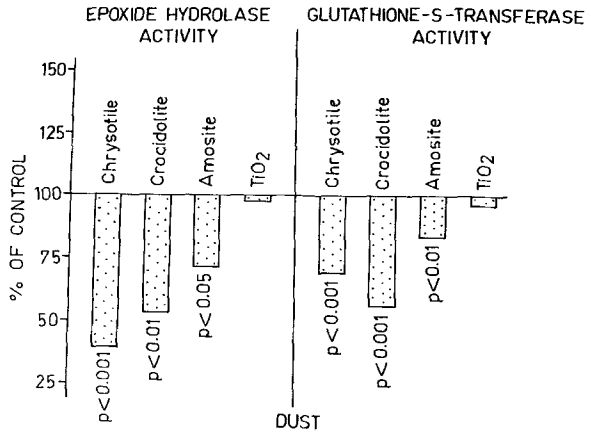

Figure 1 Effect of asbestos fibres both on the activities of microsomal epoxide hydrolase and cytosolic glutathione-s-transferase in rat lung. The details of the experimental procedure is described in the text. Each values represent percent of controls. Control values, mean ± SE (n = 6) were, epoxide hydrolase activity 0.035±0.003 fluorescence unit/mg microsomal protien/hour, glutathione-S-transferase activity 126.2±4.4 n moles CDNB conjugate formed/min/mg cytosolic protein.

mediated increase in microsomal lipid peroxidation, as observed in our previous studies (Rahman et al.,1988), may be involved in the disruption of microsomal membrane, leading to the deactivation of these proteins. This is further strengthened by the fact that these fibres show analogous differential effects on peroxidative damage to hepatocytes (Rahman and Casciano,1985). In conclusion, the results of the present study, suggest that the diminution in phase I and phase II drug metabolizing enzymes in pulmonary tissue by asbestos may increase the retention time of the reactive metabolites of the carcinogenic chemicals of cigarette smoke in lungs and thus might be contributing to some extent in the increased risk of development of lung cancer in smokers, occupationally exposed to asbestos. Further studies in this direction to support the aforesaid hypothesis are in progress.

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