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EFFECTS OF ASBESTOS ON MEMBRANE TRANSPORT AND METABOLISM OF BENZO(a)PYRENE

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SUMMARY: The effect of asbestos on benzo(a)pyrene uptake by microsomal membranes and lipid micelles has been investigated. Asbestos mediates a rapid transport of the carcinogen into the membrane and also impairs benzo(a)pyrene metabolism in rabbit and rat liver microsomes by markedly inhibiting aryl hydrocarbon hydroxylase.

INTRODUCTION

Asbestos, a naturally occurring mineral fiber which is almost ubiquitously distributed in the present day environment, is potentially hazardous to man (1). The association of occupational asbestos exposure with respiratory cancer is now well recognized (2,3). Besides being a physical carcinogen asbestos may also markedly augment the effect of other potent chemical carcinogens, such as polynuclear aromatic hydrocarbons, found in cigarette smoke (3). A vast majority of asbestos-related cancers appear to result from the cocarcinogenic effects of asbestos exposure and cigarette smoking (2).

In the induction of lung cancer it is vital that the inhaled carcinogen be retained by the lungs (4). This may be facilitated by the adsorption of the carcinogen onto asbestos, thereby retarding its pulmonary clearance, enhancing the total exposure level to the carcinogen and prolonging the duration of contact with the site of metabolic activation to ultimate carcinogens. Recent studies (5,6) have suggested that asbestos fibers adsorb carcinogenic hydrocarbons and then function as carriers for the transport of these carcinogens into cells. Metabolic activation and disposition of polynuclear aromatic hydrocarbons, such as benzo(a)pyrene, are mediated by the membrane-bound enzyme

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system, aryl hydrocarbon hydroxylase (7). Factors which modify the activity of this enzyme may have profound effects on the microsomal levels of benzo(a)pyrene. Inasmuch as asbestos particles could influence the cellular availability of benzo(a)pyrene, it would be instructive to investigate the effects of asbestos on microsomal aryl hydrocarbon hydroxylase activity. Such consideration have prompted to examine the effects of asbestos on the microsomal uptake and metabolism of benzo(a)pyrene and these results constitute the present communication.

MATERIALS AND METHODS

Benzo(a)pyrene, NADPH (type I), cis-linoleic acid, soybean lecithin (type III-S) and methemoglobin (grade I) were obtained from Sigma Chemical Company, St. Louis, MO. Standard samples of asbestos were supplied by the International Union Against Cancer, Johannesburg. Microsomal fractions were isolated from liver homogenates of rabbits and rats (Sprague-Dawley) by differential centrifugation, following standard procedures (8). Rats were treated with 3-methylcholanthrene as described by Yang et al. (9).

Aryl hydrocarbon hydroxylase activity was determined essentially as described by Yang et al. (9). The assay system contained 0.2 μmoles of EDTA and did not include $MgSO_4$. Benzo(a)pyrene (25 nmoles dissolved in acetone) was pre-incubated with 50 μl of asbestos sample (0.5 mg/ml) at 37°C for 15 minutes. Protein was estimated by the method of Lowry et al. (10) with crystalline bovine serum albumin as the standard. Benzo(a)pyrene uptake by linoleic acid was monitored fluorometrically adopting the procedure of Lakowicz and Hylden (5). A solution of benzo(a)pyrene in benzene was evaporated to dryness and then 0.1 M potassium phosphate buffer (pH 7.5) was added to the crystals and the suspension was then sonicated for 10 minutes. The aqueous suspension of benzo(a)pyrene is referred to as microcystalline. Benzo(a)pyrene uptake was measured in a system containing 0.1 M potassium phosphate buffer, pH 7.5 and 2 μM benzo(a)-pyrene in a 3 ml final volume. The concentrations of other components were as follows: linoleate (as sodium salt), 100 μM; asbestos sample 20 μl (Canadian chrysotile, 0.2 mg/ml). Benzo(a)pyrene uptake by microsomal fraction or lecithin was studied by measuring the fluorescence intensity at 405 nm (excitation wavelength, 368 nm). The reaction mixture contained 15 nmoles of benzo(a)pyrene in acetone and other additions as appropriate (i.e., 10-20 μg of microsomal protein, 8 μl of lecithin (15 mg/ml) or 20 μl of crocidolite asbestos (0.5 mg/ml) in 3 ml of 0.1 M potassium phosphate buffer, pH 7.5. Lecithin was dispersed in this buffer by sonication. Linoleate oxidation was measured with a Clark oxygen electrode. Sodium linoleate (10 mM) and, where applicable, 100 μM benzo(a)pyrene, or 40 μl of asbestos sample (Canadian chrysotile; 0.2 mg/ml), in a final volume of 2 ml of 0.1 M potassium phosphate buffer, pH 7.5 was equilibrated at 37°C and the reaction was initiated by the addition of 0.5 μM methemoglobin.

RESULTS AND DISCUSSION

Our initial study concerns the effect of asbestos on benzo(a)pyrene uptake by lipids since it has been indicated that the adsorption of benzo(a)pyrene to asbestos would enhance its transfer to phospholipid vesicles (5). When

linoleate is mixed with an aqueous suspension of benzo(a)pyrene (microcrystalline) there is an increase in its fluroescence intensity in the region of 405 nm. There appears a major peak at this wavelength with a concomitant disappearance of a broad structureless emission at higher wavelengths, designated as the excimer emission (5). The emission spectrum at this point is highly structured and is similar to that of benzo(a)pyrene dissolved in benzene. A structured fluorescence emission is indicative of widely dispersed benzo(a)pyrene molecules (5). These fluorescence changes caused by linoleic acid may be due to the solubilization of benzo(a)pyrene in the lipid phase. An even greater augmentation in the relative fluorescence intensity of benzo(a)pyrene at 405 nm is noticed when linoleic acid is added to an aqueous suspension of benzo(a) pyrene incubated with asbestos. However, no appreciable change in fluorescence is observed when asbestos is mixed with benzo(a)pyrene pretreated with linoleate. Lakowicz and Hylden (5) observed similar changes in fluorescence emission when dipalmitoyl-L-α-phosphatidylcholine was added to asbestos-adsorbed benzo(a)pyrene. These authors contend that asbestos adsorbs and disperses benzo(a)pyrene in a monomeric state facilitating its solubilization and consequently increasing its cellular availability. In the light of these observations and on the basis of our results it may be concluded that asbestos particles enhance the transport of benzo(a)pyrene into lipids and partition the carcinogen in the lipid phase.

To further substantiate the role of asbestos in transporting benzo(a)-pyrene to membranes, we have examined the effect of adsorption onto particles on the microsomal uptake of benzo(a)pyrene. Treatment of the microsomal fraction with benzo(a)pyrene, pre-incubated with asbestos at $37^{o}C$, results in a rapid increase in the fluorescence intensity of benzo(a)pyrene at 405 nm (Table 1). The observed fluoroscence is higher than that obtained when microsomal fraction is incubated with benzo(a)pyrene under the same conditions. Addition of asbestos to an incubation system, comprising

TABLE I

Effect of asbestos on the binding of benzo(a)pyrene to microsomes *

Incubation	Addition	Fluorescene intensity (%)
Benzo(a)pyrene + asbestos	--	28
Benzo(a)pyrene + asbestos	microsomes	100
Benzo(a)pyrene + microsomes	--	60
Benzo(a)pyrene + microsomes	asbestos	65
Benzo(a)pyrene + asbestos	lecithin	82
Benzo(a)pyrene + lecithin	--	65
Benzo(a)pyrene + lecithin	asbestos	60

The components specified under "Incubation" were incubated at 37°C for 5 minutes and then the flourescence intensity of the sample was recorded. To this system the material mentioned under "Addition" was added and the flourescence intensity was measured again. The asbestos sample used was crocidolite (0.5 mg/ml).

benzo(a)pyrene pre-incubated with microsomes at 37°C, does not change the fluorescence intensity significantly. From these results it may be discerned that an increased extent of benzo(a)pyrene uptake could ensue by the adsorption of the carcinogen onto asbestos. Thus asbestos would seem to influence the microsomal accessibility of benzo(a)pyrene. Lakowicz and Bevan (11) observed that adsorption of benzo(a)pyrene to particles (anthophyllite or Canadian chrysotile) resulted in a markedly increased benzo-(a)pyrene uptake by Aroclor-treated rat liver microsomes. A rigorous and long pretreatment of the particles with benzo(a) pyrene was essential for the enhanced uptake while simple mixtures of asbestos and the carcinogen did not stimulate uptake. However, in the present study, we have obtained an increased uptake of the carcinogen by following a simple and rapid procedure that involves the incubation of the particles with a buffered system containing benzo(a)pyrene at 37°C. Since benzo(a)pyrene is sparingly soluble in water it is likely that the increase in benzo(a)pyrene

TABLE II

Effect of asbestos on aryl hydrocarbon hydroxylase activity of liver microsomes.

Addition	Relative Activity (%)[a]		
	Rabbit	Rat	Rat(3-MC)c
None	100	100	100
Crocidolite	44	49	77
Canadian Chrysotile B	31	45	52
Amosite	71	62	84
Anthophyllite	74	71	63

a Expressed as a percentage of the aryl hydrocarbon hydroxylase activity of control containing no asbestos.

b Control rat liver microsomes were used.

c 3-methylcholanthrene-treated rat liver microsomes were used.

fluorescence noticed, when microsomes are added to benzo(a)pyrene-asbestos particle, is due to the solubilization and dispersal of the carcinogen in the lipid bilayer of the microsomal membrane. That this is a possibility is also indicated by similar results obtained with experiments on benzo(a)pyrene binding to lecithin (Table I). Based on these results an important role for lipids in the microsomal transport of benzo(a)pyrene may be visualized.

Adsorption of benzo(a)pyrene onto asbestos seems to have an effect not only on the microsomal uptake of benzo(a)pyrene but also on its metabolism. A perusal of Table II would indicate that asbestos causes a marked inhibition of rabbit and rat liver microsomal aryl hydrocarbon hydroxylase activities. Canadian chrysotile and crocidolite appear to be very effective inhibitors of this activity in rabbit and rat liver microsomes. Maximal enzyme inhibition is observed with chrysotile in all the microsomal fractions examined. Asbestos is also found to inhibit the hydroxylase in 3-methylcholanthrene-pretreated rat lung microsomes. Thomson et al. (12) reported that certain trace metals, considered to be associated with asbestos, inhibited benzo(a)pyrene metabolism in rat liver microsomes.

In their study trace metals were found to inhibit enzyme activity to an increasing extent as their concentrations were increased. However, we find that increasing the concentration of asbestos in the assay mixture does not result in an increase in the degree of inhibition of aryl hydrocarbon hydroxylase activity. Also the metal chelating agent, EDTA, has no effect on this inhibition. Therefore, it is unlikely that the inhibition of aryl hydrocarbon hydroxylase by asbestos is due to asbestos-associated trace metals.

The modifying effect of asbestos on aryl hydrocarbon hydroxylase activity may have important consequences *in vivo*. Tishler *et al.* (13) have suggested that the high risk of lung cancer for cigarette smokers, occupationally exposed to asbestos, is due to carcinogenic hydrocarbons of cigarette smoke, such as benzo(a)pyrene, which concentrate on the asbestos fibres, enter the body and consequently remain in the lungs. By retarding the rapid metabolism of benzo(a)pyrene, asbestos may be prolonging the retention of the carcinogen in the tissue thereby increasing the risk of induction of carcinoma.

McLemore *et al.* (6) have recently shown that aryl hydrocarbon hydroxylase activity in lymphocytes, cultured in presence of asbestos pre-incubated with benzanthracene, was much less than in cells cultured with benzathracene alone. Their results suggest that asbestos particles could store carcinogenic hydrocarbons on their surfaces. These observations are in accord with the demonstration that labelled benzo(a)pyrene remained in animal tissue for longer periods of time when it was adsorbed to asbestos and then instilled into the respiratory tract (14).

It is known that benzo(a)pyrene can inhibit the autoxidation of unsaturated lipids and unsaturated fatty acids with concurrent oxidative destruction of the carcinogen (15). It is of interest to examine whether asbestos has any effect on this property of benzo(a)pyrene. Asbestos,

incubated with benzo(a)pyrene at 37°C for 5 minutes, reverses the inhibition by benzo(a)pyrene of linoleate oxidation.

Taken together the present results suggest that adsorption of benzo(a)-pyrene onto the surface of asbestos facilitates a rapid transfer of the carcinogen to the microsomal membrane, thus increasing its accessibility to benzo(a)pyrene. The transported benzo(a)pyrene might not be readily available for its immediate metabolic disposition or for other oxidative processes. The consequences of this could, therefore, be a slower metabolic detoxification of benzo(a)pyrene and its less rapid clearance from the lungs, thus enhancing the risk of carcinoma. This would also explain the greater induction by polycyclic aromatic hydrocarbons of benzo(a)-pyrene hydroxylase in lymphocytes isolated from asbestos workers than in controls, reported by Naseem et al. (16), and confirmed by us (P.J. O'Brien, unpublished data). It may be surmised that the modifying effect of asbestos on benzo(a)pyrene metabolism could have a possible role in cocarcinogenesis.

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