Biochemical Pharmacology, Vol. 30, No. 7, pp. 811-814, 1981.
Printed in Great Britain.

0006-2952/81/070811-04$02.00/0

PRELIMINARY COMMUNICATION

PULMONARY METABOLISM OF BENZO(a) PYRENE : EFFECT OF ASBESTOS

C. Kandaswami and P.J. O'Brien

Department of Biochemistry, Memorial University of Newfoundland,
St. John's, Newfoundland A1B 3X9, Canada

(Received 10 September 1980; accepted 12 January 1981)

In recent years, there has been an intense interest in the biological effects of asbestos (1,2). This mineral fiber, almost ubiquitously distributed in the present day environment, is widely recognized as a major occupational health hazard (3,4). Of particular concern in this context is the association of asbestos inhalation with lung cancer. While the main etiologic agent in the causation of pulmonary carcinoma is cigarette smoke, several other exogenous factors, including environmental chemicals, also seem to be involved (4). Occupational exposure to asbestos is known to increase the risk of bronchogenic carcinoma. Pathological, epidemiological and experimental studies all show that simultaneous exposure to polycyclic aromatic hydrocarbons, known to be present in cigarette smoke, and asbestos results in synergistic or cocarcinogenic effects (3,4). For instance, asbestos workers who smoke cigarettes have a markedly increased risk of carcinoma when compared to either non-smokers or smokers in the general population. Epidemiological studies suggest that cocarcinogenic effects of asbestos exposure and cigarette smoking are responsible for the majority of the cancers resulting from asbestos exposure.

In the induction of pulmonary carcinoma the retention of the inhaled carcinogen in the tissue would seem to be essential (5). Since asbestos is known to be efficiently retained in the lungs (6), the adsorption of the carcinogen to the particulate may retard the pulmonary clearance of the carcinogen, thus enhancing its exposure level. The metabolism of carcinogenic hydrocarbons, such as benzo(a)pyrene, is mediated by the membrane-bound enzyme system, aryl hydrocarbon hydroxylase (7). Factors which modify this activity may have a marked effect on the metabolic disposition of benzo(a)pyrene. Recent studies in our laboratory (8) have indicated that asbestos could impair benzo(a)pyrene metabolism in isolated rabbit and rat liver microsomal fractions. In the present communication we report the effect of asbestos on aryl hydrocarbon hydroxylase activity of rat lung microsomes.

EXPERIMENTAL PROCEDURES

Chemicals. Benzo(a)pyrene, NADPH (type I) and 3-methylcholanthrene were obtained from Sigma Chemical Company, St. Louis, MO., U.S.A. All other chemicals and reagents used were of analytical grade available commercially. Standard samples of asbestos were supplied by the International Union Against Cancer, Johannesburg.

Animals and Treatment. Male Sprague-Dawley rats weighing between 200 and 250 g were used in these experiments. Animals were pre-treated by a daily i.p. injection of 3-methylcholanthrene (in corn oil, 40 mg/kg body weight) for 4 days. They had free access to laboratory chow and drinking water.

Preparation of Microsomes. Lung microsomal fraction was isolated by following essentially the procedure of Matsubara *et al.* (9).

Assays. Aryl hydrocarbon hydroxylase was assayed as described by Yang et al. (10). The reaction mixture in a final volume of 1 ml, containing 100 µmoles of potassium phosphate buffer (pH 7.5), 0.2 µmole of EDTA, microsomal fraction (0.2 to 0.4 mg of protein) and asbestos sample (2 mg), was preincubated at 37°C for 1 hour. Thereafter 40 nmoles of benzo(a)pyrene (dissolved in acetone) was added to the incubation mixture and the incubation was continued for another 2 minutes. Finally the reaction was initiated by the addition of 0.4 µmole of NADPH. Alternatively, in some experiments 40 nmoles of benzo(a)pyrene was preincubated with 50 µl of asbestos sample (an aqueous suspension containing 0.5 mg of asbestos per ml) for 10 minutes at 37°C. The microsomal fraction was then added to this mixture and the incubation was continued for another 5 minutes. Thereafter, the reaction was started by the addition of NADPH. In either case, the reaction was allowed to proceed for 5 minutes and was terminated by the addition of 4 ml of acetone-hexane (1:3, v/v). A 2-ml aliquot of the organic phase was extracted with 4 ml of 1N NaOH. The flourescence of the phenolic products in the aqueous phase was measured in a Perkin-Elmer flourescence spectrophotometer with activation at 396 nm and emission at 522 nm. Protein was estimated by the method of Lowry et al. (11) with crystalline bovine serum albumin as the standard.

RESULTS AND DISCUSSION

As shown in Table I, treatment of lung microsomal fraction with asbestos resulted in a marked diminution of aryl hydrocarbon hydroxylase activity. Of the different asbestos fibers examined crocidolite caused the least inhibition of the enzyme.

Table I. Effect of asbestos on aryl hydrocarbon hydroxylase activity of rat lung microsomes

Addition [a]	Relative activity (%) [b]
None	100
Crocidolite	34 (32-36)
Canadian Chrysotile B	46 (42-50)
Amosite	38 (35-41)
Anthophyllite	40 (36-43)

[a] Asbestos was preincubated with the microsomal fraction for 1 hour as described in the Experimental Procedures.

[b] Expressed as a percentage of the aryl hydrocarbon hydroxylase activity of the control containing no asbestos. The specific activity of the control was 15 pmoles/minute/mg of protein. The values represent averages of 3 separate experiments. The range is given within parentheses.

Pre-incubation of benzo(a)pyrene with asbestos also depressed the microsomal aryl hydroxylase activity (Table II). In this case however, relatively small amounts of asbestos were found to inhibit this activity. The present observations thus suggest that asbestos could prevent benzo(a)pyrene from being metabolized in the tissue. These results are in accord with the report of Pylev et al. (12), that labelled benzo(a)pyrene remains in animal tissue for longer periods of time in the presence of asbestos.

The aryl hydrocarbon hydroxylase system is of major importance in the regulation of the microsomal biotransformation of polycyclic aromatic hydrocarbons (7,13). It is associated

Table II. Effect of pretreatment of benzo(a)pyrene with asbestos on aryl hydrocarbon hydroxylase activity of rat lung microsomes

Addition [a]	Relative activity (%) [b]
Control	100
Crocidolite	58
Canadian Chrysotile B	64

[a] Benzo(a)pyrene was pretreated with asbestos as described in the Experimental Procedures.

[b] Expressed as a percentage of the activity of the control containing no asbestos. The values are averages of 3 separate experiments.

with the detoxification or activation of these hydrocarbons in chemical carcinogenesis. High levels of this enzyme result in rapid metabolism of these carcinogens to non-carcinogenic metabolites (7,14). The inhibitory effect of asbestos on aryl hydrocarbon hydroxylase could have important consequences *in vivo*. Interference of asbestos with the detoxification of benzo(a)pyrene may result in a slower pulmonary clearance of the carcinogen, thereby increasing its retention time. With asbestos, large amounts of material are retained in the tissues unlike other carcinogenic agents which are excreted or metabolized (6). Asbestos appears to be capable of adsorbing benzo(a)pyrene and other hydrocarbons (15-18). If asbestos were to retard the detoxification of benzo(a)pyrene, the presence of residual retained asbestos may ensure a continuing exposure of the tissue to the carcinogen. Thus, benzo(a)pyrene may remain biologically active in the lung for a prolonged duration. The retention of unmetabolized benzo(a)pyrene in the lungs would accentuate the carcinogenic stimulus insofar as the risk of bronchogenic carcinoma correlates with the duration of exposure to the carcinogen (5). Carcinogenesis with polycyclic aromatic hydrocarbons requires the persistence of non-metabolized carcinogen at the site of administration or selective localization (19). It has been demonstrated that safrole, a hepatic carcinogen, interferes with the hepatic detoxification and elimination of benzo(a)pyrene (20). Diminished pulmonary clearance and hydroxylation of benzo(a)pyrene have also been observed following administration of nickel carbonyl to rats (21). On the basis of this work it has been suggested that nickel might promote pulmonary carcinogenesis by inhibiting benzo(a)pyrene hydroxylase and thus prolonging the tissue retention of benzo(a)pyrene.

Certain asbestos-associated trace metals have been shown to cause a concentration-dependent change in the rate of metabolism of benzo(a)pyrene in rat liver microsomes and rat lung homogenates (22,23). The inhibition of aryl hydrocarbon hydroxylase activity by asbestos, observed presently with lung microsomes, does not seem to be due to trace metals associated with asbestos. The metal chelating agent, EDTA, has no effect on the inhibition of the hydroxylase by asbestos. EDTA, however, is found to protect the enzyme against inhibition by Cu^{2+}.

Acknowledgement: This work was supported by grants from the National Cancer Institute of Canada.

REFERENCES

1. D. Shugar, Effects of Asbestos in the Canadian Environment, Publication No. NRCC 16452 of the Environmental Secretariat, National Research Council of Canada, Ottawa, Canada (1979).
2. Q. Rahman, P.N. Viswanathan and S.H. Zaidi, Environ. Res. 14, 487 (1977).
3. L. Preger, in Asbestos-related Disease (Eds. L. Preger, D.T. Arai, P. Kotin, H. Weill and D. Werchick), p. 174. Grune & Stratton, New York (1978).
4. C.C. Harris, in Lung Cancer: Clinical Diagnosis and Treatment (Ed. M.J. Strauss), p. 1. Grune & Stratton, New York (1977).
5. L.M. Shabad, L.N. Pylev and T.S. Kolesnichenko, J. Natl. Cancer Inst. 33, 135 (1964).
6. H. Seidman, I.J. Selikoff and E.C. Hammond, Ann. N.Y. Acad. Sci. 330, 61 (1979).
7. W.F. Benedict, J.E. Gielen and D.W. Nebert, Int. J. Cancer 9, 435 (1972).
8. C. Kandaswami and P.J. O'Brien, Biochem. Biophys. Res. Commun. (in press).
9. T. Matsubara, R.A. Prough, M.D. Burke and R.W. Estabrook, Cancer Res. 34, 2196 (1974).
10. C.S. Yang, F.S. Strickhart and L.P. Kicha, Biochem. Pharmacol. 27, 2321 (1978).
11. O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. Biol. Chem. 193, 265 (1951).
12. L.N. Pylev, F.J.C. Roe and G.P. Warwick, Br. J. Cancer 23, 103 (1969).
13. K. Burki, A.G. Liebelt and E. Bresnick, J. Natl. Cancer Inst. 50, 369 (1973).
14. R.E. Kouri, R.A. Salerno and C.E. Whitmore, J. Natl. Cancer Inst. 50, 363 (1973).
15. J.S. Harrington, Ann. N.Y. Acad. Sci. 132, 31 (1965).
16. L.M. Shabad, L.N. Pylev, L.V. Krivosheeva, T.F. Kulagina and B.A. Nemenko, J. Natl. Cancer Inst. 52, 1174 (1974).
17. J.R. Lakowicz and J.L. Hylden, Nature (London) 275, 446 (1978).
18. T.K. McLemore, W.T. Jenkins, M.S. Arnott and N.P. Wray, Cancer Letters 7, 171 (1979).
19. L.M. Shabad, L.N. Pylev and T.S. Kolesnichenko, Federation Proc. 24, T-717 (1965).
20. H.L. Falk, S.J. Thompson and P. Kotin, Federation Proc. 25, 637 (1966).
21. F.W. Sunderman, Cancer Res. 27, 950 (1967).
22. R. Thomson, I. Webster and T.A. Kilroe-Smith, Environ. Res. 7, 149 (1974).
23. J.R. Dixon, D.B. Lowe, D.E. Richards, L.J. Cralley and H.E. Stokinger, Cancer Res. 30, 1068 (1970).