Biochemical Pharmacology, Vol. 31, No. 4, pp.607-608, 1982.
Printed in Great Britain.

0006-2952/82/040607-02$03.00/0

A FREE RADICAL MECHANISM FOR ARYLAMINE INDUCED CARCINOGENESIS INVOLVING PEROXIDES

S. Vasdev, Y. Tsuruta, and P.J. O'Brien

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

(Received 22 September 1981; accepted 19 November 1981)

Whilst much emphasis has been placed in chemical carcinogenesis on the 2 e oxidation catalytic activity of monooxygenase, it is clear that 1 e oxidation pathways mediated by prostaglandin synthetase activity, lipid peroxidation or monooxygenase activity can also form metabolites which readily bind to the informational macromolecules and could be a critical step in the initiation of neoplasia. The 1 e oxidation pathway is more active in catalysing this binding with phenols, amines and hydrazines and could explain the necrosis or carcinogenesis induced by acetaminophen, diethylstilbestrol, methylhydrazine, benzidine, benzene, hair dyes, cyclophosphamide (1). It is widely held that the ultimate carcinogen is an electrophile however the 1 e oxidation pathway forms free radicals and chemical carcinogenesis mechanisms need to be re-examined.

The metabolic N-hydroxylation of carcinogenic arylamines and arylamides are alleged to be an obligatory step in the activation of these compounds to carcinogenic derivatives and that subsequent esterification, catalysed by a liver sulfotransferase, leads to the formation of the ultimate carcinogen in the liver. Liver DNA adducts following in vivo administration of N-methyl-4-aminoazobenzene (MAB) could be explained this way (2,3). However in other tissues the lack of sulfotransferase makes this pathway less attractive. In the following a 1 e oxidation of MAB can be shown to catalyse more binding than the 2 e monooxygenase mechanism even in the presence of the liver sulfotransferase.

C^{14} MAB Binding

MAB-(ring C^{14}) was synthesized as described (4) and had a specific activity of 0.54 mCi/nmol with a purity $\geq$ 99%. The incubation mixture contained in a final volume of 2.0 ml: Tris-HCl buffer 0.1 M (pH 7.4) microsomal protein (2 mgm), NADPH (0.4 mM) or cumene hydroperoxide (1.5 mM) or arachidonate (100 μM). When binding to DNA, 2 mgm of DNA was added to the incubation mixture. Following incubation in a metabolic shaker for 15 mins. at 37°C, four extractions with 5 ml diethylether was carried out. The purification of protein or DNA was performed as described (5) and counted in aquasol in a scintillation counter.

Product Analysis

The ether extracts were evaporated, dissolved in ethanol and analysed by high performance liquid chromatography monitored at 254 nm on a μ Bondapak C18 reversed phase column using a 50-100% linear methanol gradient at a flow rate of 1 μl/min at 20°C.

RESULTS AND DISCUSSION

Prostaglandin synthetase activity of the microsomes of pig bladder, sheep vesicular gland or lung microsomes catalyses the binding of methylaminoazobenzene to exogenous DNA and microsomal protein (6). Some mixed function oxidase activity with NADPH was apparent with pig bladder or rat lung microsomes but was twenty fold lower than that with arachidonate (6). Hydroperoxides have previously been shown to catalyse cytochrome P450 function and can substitute for NADPH, O_2 and reductase in the monooxygenase activity (7). As can be seen from the table hydroperoxide was almost 400 times more effective than NADPH in catalysing the binding. This suggests that NADPH/reductase reduces the radical formed by the 1 e oxidation pathway of cytochrome P450. Addition of cytosol did not appreciably affect the hydroperoxide catalysed binding but markedly enhanced the NADPH system. As was previously shown (8) the latter cytosol effect was further enhanced by ATP and sulfate indicating that the cytosol effect was likely due to sulfotransferase acting on the NOH MAB formed by 2 e oxidation pathway carried out by microsomal amine oxidase.

COVALENT BINDING OF (^{14}C) MAB METABOLITE(S) TO EXOGENOUS DNA OR MICROSOMAL PROTEIN

Additive to rat liver microsomes	nmol/g DNA	nmol/g Protein
None	10	20
+ NADPH	60	120
+ NADPH + cytosol	650	620
+ NADPH + cytosol + ATP/SO_4	12,780	3,700
+ Hydroperoxide	15,100	6,800
+ Hydroperoxide + cytosol	10,100	5,800
+ Hydroperoxide + cytosol + ATP/SO_4	13,830	6,300

No NOH MAB is formed with hydroperoxide (9) and this explains why cytosol did not stimulate. Hydroperoxide was however similar to NADPH in the rate of the oxidation of MAB. The products as measured by HPLC were also similar and included aminoazobenzene, 4 OH methylaminoabenzene and a trace of

4 OH aminoazobenzene. This indicates that cytochrome P450 catalyses both N demethylation and ring hydroxylation of methylaminoazobenzene and that the N hydroxylation is catalysed by microsomal amine oxidase. Prostaglandin synthetase system oxidised methylaminoazobenzene (6). The binding of (^{14}C)-MAB to exogenous DNA and protein catalysed by the prostaglandin synthetase-arachidonate or cytochrome P450-hydroperoxide system clearly does not involve NOH MAB as an intermediate. The amine radical is probably responsible for the DNA/protein binding formed by the amine acting as a peroxidase donor in the same way as benzidine, B naphthylamine (10), and phenylenediamines (7). Prostaglandin synthetase or the 1 e pathway of the monoxygenase activity of bladder or lung could be responsible for bladder carcinogenesis in dog or lung carcinogenesis in mice following the administration of p-dimethylamino-azobenzene IARC 1976. Both 1 e and 2 e pathways should however be considered for liver carcinogenesis in rats as it is likely that the MAB radicals form the same DNA adducts as observed in vivo or with the NOH ester.

This work was supported by the National Cancer Institute of Canada and was presented at the First European Research Conference on "Peroxides in Biological Systems", Otzenhausen, Germany, September 7-11, 1981.

REFERENCES

1. O'Brien, P.J. (in press) "Lipid Peroxides in Biology and Medicine", Ed. K. Yagi, Academy Press, N.Y.
2. Tarpley, W.G., Miller, J.A., and Miller, E.C. (1980) Cancer Res. 40, 2493-2499.
3. Beland, F.A., Kadlubar, F.F., and Evans, F.E. (1980) Chem. Biol. Interacns. 31, 1-17.
4. Meunier, M. and Chauveau, J. (1970) Int. J. Cancer 6, 463-469.
5. Sivarajah, K., Anderson, M.W. and Eling, T.E. (1978) Life Sciences 23, 2571-2578.
6. O'Brien, P.J. (in press) "Prostaglandins and Cancer: Ed. R. Bockman, K.V. Honn, T.J. Powles and P. Ramwell, (Publ. A.R. Liss, Inc., N.Y.).
7. O'Brien, P.J. (1978) Pharm. Ther. 2A, 517-536.
8. Labuc, G.E. and Blunck, J.M. (1979) Biochemical Pharm. 28, 2367-2373.
9. Kadlubar, F.F., Miller, J.A. and Miller, E.C. (1976) Cancer Res. 36, 1196-1206.