Vol. 94, No. 3, 1980 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
June 16, 1980 Pages 737-743

GENETIC DEPENDENCE OF HEPATIC MICROSOME-MEDIATED DEPRESSION OF AFLATOXIN B_1 ACTIVATION TO MUTAGENS IN AMES SALMONELLA TYPHIMURIUM TA-98 SYSTEM[1]

V. Raina, H.L. Gurtoo[2], M. Berrigan and B. Paigen

Department of Experimental Therapeutics and
Grace Cancer Drug Center (VR, HLG, MB), and
The Department of Molecular Biology (BP)
Roswell Park Memorial Institute
Buffalo, New York 14263

Received March 26,1980

SUMMARY:

Pretreatment of C57Bl/6, an Ah-responsive strain of mice, with 3-methyl cholanthrene or β-naphthoflavone caused a depression of the hepatic microsome-mediated metabolism of alfatoxin B_1 to metabolites mutagenic to Salmonella thyphimurium TA-98 strain, while the activity of hepatic mecrosomes from an Ah nonresponsive strain DBA/2 was unaffected by these pretreatments. In contrast to results obtained with aflatoxin B_1, 3-methylcholanthrene or β-napthoflavone pretreatment of C57Bl/6 enhanced several fold the metabolism of benzo(a)pyrene to mutagenic metabolites in this system, whereas pretreatment of DBA/2, like that seen with aflatoxin B_1, did not affect the formation of mutagenic metabolites of benzo(a)pyrene.

INTRODUCTION:

Aflatoxin B_1 (AFB_1), a mold metabolite produced by certain strains of Aspergillus flavus and Aspergillus parasiticus, is a potent hepatotoxic and hepatocarcinogenic agent in a number of animal species (1,2). It contaminates several foods (3,4) and has been implicated in the etiology of human liver disease (5,6). Earlier reports have shown that AFB_1 is not the ultimate

[1]This work was supported by Grants CA-25362 and CA-18542 from the National Cancer Institute, U.S. Department of Health, Education and Welfare, and a Grant BC-303 from the American Cancer Society.

[2]To whom requests for reprints should be addressed.

0006-291X/80/110737-07$01.00/0

carcinogen but requires metabolic activation by microsomal mixed function oxygenases to a highly reactive metabolite (7-10). Microsome mediated-metabolism of AFB_1 to DNA-binding metabolite(s) is induced by phenobarbital(PB) pretreatment of animals but not by 3-methylcholanthrene (3MC) pretreatment (11), indicating that cytochrome P450 and not cytochrome P-448 (P_1-450) is involved in the metabolism of AFB_1 to aflatoxin B_1 - 2,3-oxide which accounts for 80-90% of AFB_1 bound in vitro to DNA (9).

Carcinogenic polycyclic aromatic hydrocarbons such as benzo(a)pyrene also require metabolic activation prior to tumor formation. These compounds are preferentially metabolized at the microsomal level by cytochrome P-448 (P_1-450)-associated mixed function oxygenases (12-14).

Earlier genetic studies of aflatoxin B_1 metabolism from our laboratory (15) have shown that 3MC pretreatment causes induction of alfatoxin B_1 -4-hydroxylase activity in some inbred strains of mice, leading to the formation of aflatoxin M_1 (AFM_1); the strains of mice inducible for aflatoxin B_1 -4-hydroxylase activity were also inducible for aryl hydrocarbon hydroxylase activity (AHH) (15). Additional investigation in recombinant inbred (RI) lines and progeny of appropriate crosses showed that the induction of AFM_1 formation, like that of AHH, is regulated in C57Bl/6 and DBA/2 mice by a single gene locus with two alleles, one dominant and the other recessive. As a consequence, strong association between the induction of AFM_1 formation and AHH was observed in 900 mice of different inbred strains, RI lines and progeny of appropriate crosses (15). These investigations suggested that regulation of the induction of both the nuclear and microsomal aflatoxin B_1-4-hydroxylase(s) and its association with the induction of aryl hydrocarbon hydroxylase occur at the level of common regulatory genetic factors.

In subsequent studies, it was demonstrated that while the formation of the DNA-binding metabolite represents an activation process (16), the formation of AFM_1 is essentially a detoxification process since AFM_1 or its further metabolites were less than 5% as active as AFB_1 in Ames mutagenesis system (16,17). These investigations have led to an interesting hypothesis that if benzo(a)pyrene

and AFB_1 are activated by different cytochrome P450's with different induction specificities, induction of AFM_1 forming enzyme should afford protection against AFB_1. This suggestion has important implications in environmental carcinogenesis where people are exposed to more than one carcinogen. Humans in certain underdeveloped countries (3,4), where exposure to high levels of aflatoxins is common, may be simultaneously exposed to polycyclic aromatic hydrocarbons (PAH), e.g., smokers, cooks, coke oven workers, and others. Thus, exposure to one carcinogen could induce a detoxifying system for another, in this case, more potent carcinogen. Because of these considerations, it was of interest to investigate the genetic dependence of interaction between AFB_1 and benzo(a)pyrene in mutagenesis.

In the present study, in vitro hepatic microsome-mediated mutagenesis of AFB_1 in Ames bacterial test system (TA-98) has been studied, using microsomes from inducible and non-inducible strains of mice, and the results compared with the mutagenesis of benzo(a)pyrene.

MATERIALS AND METHODS

Chemicals:

Chemicals were obtained as follows: benzo(a)pyrene from Aldrich Chemical Company; AFB_1 from Calbiochem.; NADPH and menadione from Sigma Chemical Company. Bacterial tester strain TA-98 Salmonella typhimurium was obtained from Dr. B.N. Ames, University of California, Berkeley, California. DBA/2 and C57Bl/6 female mice (between 6-7 weeks of age) were obtained from the animal facilities of our departments.

Hepatic microsomes were isolated as previously reported (15,16). All the steps were carried out at 4°C using sterile glassware and cold sterile solutions. Pretreatment with 3MC and β-naphthoflavone (βNF) consisted of a single intraperitoneal injection of 3MC (60 mg/kg) or βNF (150 mg/kg) in corn oil 48 hours before sacrifice. Control mice received corn oil only.

The basic mutagenesis assay of Ames and coworkers (18,19) was modified by preincubating in a buffer mixture 0.1 ml of an overnight culture of the bacterial strain S. typhimurium TA-98, NADPH, benzo(a)pyrene or AFB_1 and microsomal protein from control or induced mice. The reaction was terminated at the end of 5 min incubation period by the addition of menadione, an inhibitor of cytochrome P-450 associated electron transport system (20).

RESULTS AND DISCUSSION

The effect of varying concentrations of menadione was studied on the mutagenesis of benzo(a)pyrene by adding menadione at either zero minutes or five

minutes after the incubation. Figure 1(A) shows that 108 nmoles menadione completely blocked the formation of mutagenic metabolites of benzo(a)pyrene when added at zero minutes, but not when added at the end of five minutes incubation. These results demonstrate that mutagenic metabolites of benzo(a)pyrene were formed during the five minute period of incubation. Similar results were obtained when aflatoxin B_1 was used as a mutagen. For subsequent experiments 108 nmoles of menadione was used to terminate the reaction. Each control and test incubations were run in duplicate and bacterial colonies were scored after 48 hours of incubation at 37°.

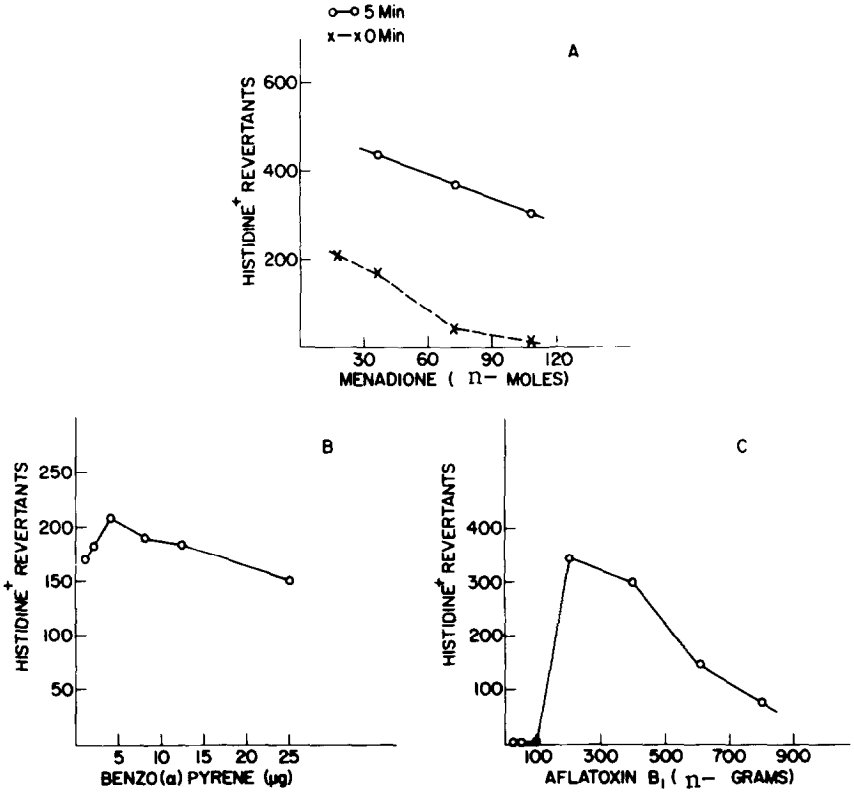

Figure 1A: Effect of varying concentrations of menadione on the mutagenesis of benzo(a)pyrene using hepatic microsomal proteins from 3MC pretreated C57B1/6 mice (protein concentration was 0.5 mg/incubation).

Figure 1B: Dose response curve for benzo(a)pyrene mutagenesis, using hepatic microsomes from 3MC pretreated C57B1/6 mice (protein concentration was 0.5 mg/incubation).

Figure 1C: Dose response curve for AFB_1 mutagenesis using hepatic microsomes from corn oil pretreated C57B1/6 mice (protein concentration was 0.5 mg/incubation).

As illustrated in Figure 1 (B), the highest number of revertant colonies occured between 4-10 µg benzo(a)pyrene; increasing concentrations of benzo(a)pyrene resulted in fewer revertant colonies. With AFB_1 the highest frequency of revertants was observed at 200 ng(1C).

As shown in Figure 2(A), the formation of the mutagenic metabolites of benzo(a)pyrene by hepatic microsomes from Ah-nonresponsive strain (DBA/2) was not affected by pretreatment of the animals with either 3MC or βNF, and also the number

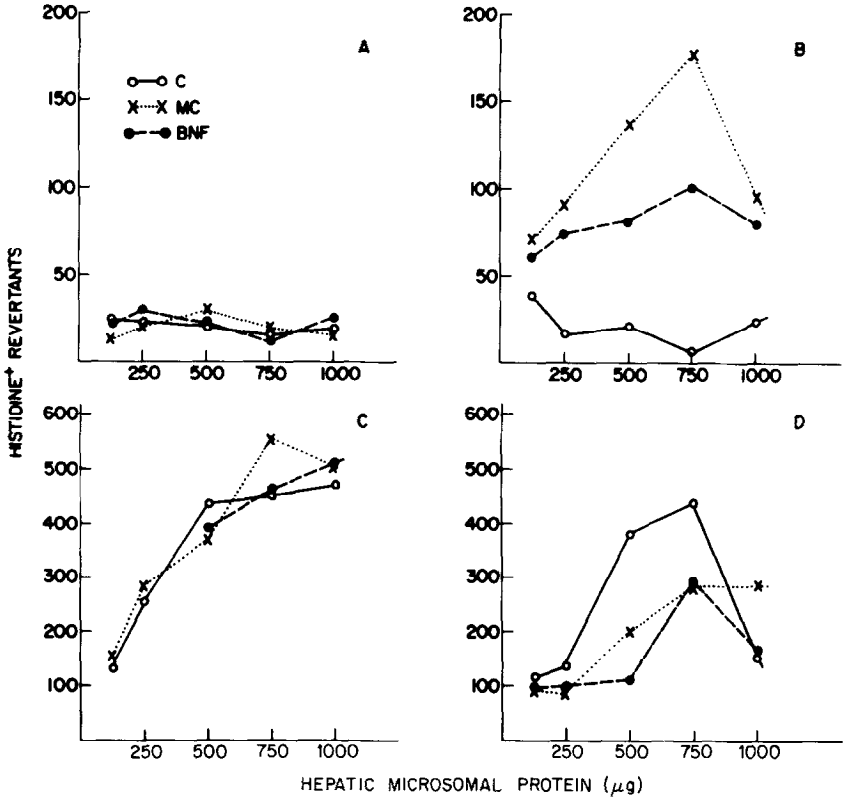

Figure 2A: Effects of different concentrations of hepatic microsomes from corn oil (C), βNF or 3MC pretreated DBA/2 mice on the mutagenesis of benzo(a)pyrene (benzo(a)pyrene concentration was 5 µg/incubation).

Figure 2B: Effects of different concentrations of hepatic microsomes from corn oil (C), βNF or 3MC pretreated C57B1/6 mice on the mutagenesis of benzo(a)pyrene (benzo(a)pyrene concentration was 5 µg/incubation).

Figure 2C: Effects of different concentrations of hepatic microsomes from corn oil (C), βNF or 3MC pretreated DBA/2 mice on the mutagenesis of AFB_1 (AFB_1 concentration was 200 ng/incubation).

Figure 2D: Effects of different concentrations of hepatic microsomes from corn oil (C), βNF or 3MC pretreated C57B1/6 mice on the mutagenesis of AFB_1 (AFB_1 concentration was 200 ng/incubation).

of revertants did not increase between 250 µg and one mg microsomal protein in the incubation. On the other hand, the number of revertants formed in the presence of benzo(a)pyrene was enhanced several fold when hepatic microsomes from Ah-responsive mice (C57Bl/6) pretreated with either 3MC or βNF were used. In the presence of these microsomal preparations, the number of revertants formed was dependent upon the concentration of microsomal protein. It increased between 125-750 µg protein but decreased when one mg microsomal protein was used.

When AFB_1 was used as substrate in the mutagenesis assay (Figure 2C and D), mutation frequency was several fold higher than that obtained with benzo(a)pyrene, irrespective of the source of the microsomes. Furthermore, the number of revertants formed was dependent upon the concentration of the microsomal protein. In the presence of hepatic microsomes from DBA/2, the number of mutants formed generally increased between 125 µg and 1 mg protein (Figure 2C). On the other hand, highest number of mutants was obtained with 750 µg, rather than one mg, of hepatic microsomal protein from C57Bl/6 (Figure 2D). Pretreatment of DBA/2 (an Ah-nonresponsive strain) mice with either 3MC or βNF had no effect on the number of mutants formed at various concentrations of the microsomal protein (Figure 2C). On the other hand, pretreatment of C57Bl/6 (an Ah-responsive strain) mice with either 3MC or βNF substantially depressed the formation of mutagenic metabolites of aflatoxin B_1 in the concentration range of 125 to 750 µg microsomal protein (Figure 2D). These contrasting effects of 3MC (and βNF) pretreatment of C57Bl/6 on the formation of the mutagenic metabolites of benzo(a)pyrene and AFB_1 are consistent with an earlier report from this laboratory (15). In this report, it was demonstrated that pretreatment of Ah responsive mice with 3MC depresses the metabolism of AFB_1 to the DNA-binding metabolite, presumably AFB_1-2,3,-oxide (9), but enhances the detoxification of AFB_1 via the formation of AFM_1. The present data can be interpreted to suggest that exposure to chemicals which induce benzo(a)-pyrene metabolism may afford some protection against a more potent carcinogen AFB_1, by inducing a pathway for its detoxification.

Several common vegetables belonging to Brassicaceae family (e.g., cabbage, cauliflower, brussel sprouts, broccoli and turnips) are known to induce benzo(a)-pyrene metabolism (21). In countries where aflatoxin contamination of foods is rampant, consumption of these vegetables may afford some protection against aflatoxin carcinogenesis; this speculation derived from the present experimental data on mutagenesis is, however, based on the assumption that a good correlation exists between carcinogenesis and mutagenesis. In any case, it would be interesting to determine whether these vegetables protect laboratory animals against aflatoxin carcinogenesis.

REFERENCES:

1. Newberne, P.M. and Butler, W.H. (1969) Cancer Res. 29: 236.
2. Wogan, G. N. Edwards, G.S. and Newberne, P.M. (1971) Cancer Res., 31: 1936.
3. Campbell, T.C, Caedo-Jun., J.P., Bultao-Jayme,J. and Engel, R.L. (1970) Nature, 227: 403.
4. Peers, F.B. and Linsell, C.A. (1973) Brit. J. Cancer, 27: 473.
5. Macsween, R.N.M. and Scott, A.R. (1973) J. Clin. Pathol, (Lond), 26: 936.
6. Vogel, C.L., Anthony, P.P., Mody, N. and Baker, L.F. (1970) Lancet., 2: 621.
7. Garner, R.C., Miller, E.C. and Miller, J.A. (1972) Cancer Res., 32: 2058.
8. Gurtoo, H.L. and Dave, C.(1973) Res. Commun. Chem. Pathol. Pharmacol., 5: 635.
9. Essigmann, J.M., Croy, R.G., Nadzan, A.M., Busby, W.F., Jr., Reinhold, V.N., Buchi, G. and Wogan, G.N. (1977) Proc. Natl. Acad. Sci. U.S.A., 74: 1870.
10. Lin, J-K., Miller, J.A. and Miller, E.C. (1977) Cancer Res., 37: 4430.
11. Gurtoo, H.L. and Bejba, N. (1974) Biochem. Biophys. Res. Commun., 61: 735.
12. Nebert, D.W., Robinson, J.R., Niwa, A., Kumaki, K. and Poland, A.P. (1975) J. Cell Physiol., 85: 393.
13. Borgen, A., Dervey, H., Castagnoli, N., Crocker, T.T., Rasmussen, R.E. and Yang, Y.T. (1973) J. Med. Chem., 16: 502.
14. Yang, S.K., McCourt, D.W., Leutz, J.C. and Gelboin, H.V. (1977) Science, 196: 1199.
15. Gurtoo, H.L., Dahms, R.P., Kanter, P.M. and Vaught, J.B. (1978) J. Biol. Chem., 253: 3952.
16. Gurtoo, H.L., Dahms, R.P. and Paigen, B. (1978) Biochem. Biophys. Res. Commun., 81: 965, 1978.
17. Wong, J.J. and Hsieh, D.P.H. (1976) Proc. Natl. Acad. Sci. U.S.A., 73: 2241.
18. Ames, B.N., Durston, W.E., Yamasaki, E. and Lee, F.D. (1973) Proc. Natl. Acad. Sci. U.S.A., 70: 2281.
19. Ames, B.N., McCann, J. and Yamasaki, E. (1975) Mutation Res., 31: 343.
20. Thakker, D.R., Yagi, H., Akagi, H., Koreeda, M.,Lu, A.Y.H., Levin, W., Wood, A.W. Conney, A.H. and Jerina, D.M. (1977) Chem.-Biol. Interactions, 16: 281.
21. Wattenberg, L.W., Loub, W.D., Like, K.L. and Speier, J.L. (1976) Fed. Proc., 35: 1327.