EVIDENCE OF PARTIAL INVOLVEMENT OF P-450 2D IN MUTAGENIC ACTIVATION OF BENZO(a)PYRENE IN LIVER S-9 FRACTION FROM UNTREATED RATS

Makihiko MASUDA, Chiyoko NUKUZUMA, Akio KAZUSAKA and Shoichi FUJITA

Department of Toxicology, Faculty of Veterinary Medicine, Hokkaido University, Sapporo 060, Japan

Received July 25, 1994; Accepted October 20, 1994

ABSTRACT — In order to clarify which species of cytochrome P-450 is involved in activation of benzo(a)pyrene (BP) in untreated rat liver, strain and sex differences in the ability of rat liver 9000 g supernatant (S-9) to mutagenically activate BP was investigated using Ames test. The numbers of histidine revertants in Ames test after pre-incubation of TA 98 strain of Salmonella typhimurium and BP with liver S-9 from male rats were markedly higher than those obtained using female rats. In addition, a marked strain difference (Wistar > DA) in the ability of liver S-9 from Wistar and DA rats to activate BP was observed. Antibody against cytochrome P-450 2D inhibited up to 50% of the revertant formation by the activation of BP with liver S-9 from male Wistar rats. These results indicate the partial involvement of cytochrome P-450 2D subfamily as well as cytochrome P-450 species specific to male rats in activation of BP to ultimate mutagen in untreated rat liver.

KEY WORDS : CYP2D, Benzo(a)pyrene, Mutagenesis, P-450.

Carcinogenic or mutagenic activation of benzo(a)pyrene (BP) by liver enzyme system has been extensively studied (Thakker et al., 1985) and the bay region hypothesis has been proposed for the formation of the ultimate mutagen (Jerina et al., 1976). For these studies, Ames test (Ames et al., 1975) with pre-incubation of the test strain of bacteria and BP with the liver S-9 from cytochrome P-450 inducer-treated rats was often used (Wood et al., 1976). Cytochrome P-450 1A1, the polycyclic aromatic hydrocarbon induced species of P-450, effectively activates BP (Thakker et al., 1976). However, in the actual exposure situation, the animals or a man exposed to BP may or may not be in the induced state at the time of exposure. Although the activity is smaller, untreated rat liver S-9 can activate BP.

Question arises then, whether P-450 1A1 present in minute quantity in non-induced liver engages in this activation, or some other constitutive form(s). Our previous study using anti-P-450 1A1 IgG showed that involvement of P-450 1A1 in activation of BP by liver S-9 from untreated rats is negligible (Fujita et al., 1988). Ohgiya et al. purified a form of P-450 (P-450 B(a)P) which catalyzed hydroxylation of BP and inferred the possible involvement of this form of cytochrome P-450 in mutagenic activation of BP in non-induced rat liver (Ohgiya et al., 1989). They stated that P-450 B(a)P is likely to be P-450 2C11 as judging from N-terminal amino acid sequence and electrophoretic characteristics.

To this date no further investigation was carried out to test this important question: what activates BP in non-induced animals? Therefore, in this study, we searched for the species of
P-450 involved in BP activation in livers of untreated rats. To obtain some insight into this, we first investigated sex- and strain-differences in BP activation in livers of rats.

Liver S-9 fractions were prepared from 3-methylcholanthrene (3-MC) treated (40 mg/kg/day for 4 days) and untreated Wistar rats (6 weeks of age, 120–130 g) and from DA rats (6 weeks of age, 110–120 g). Protein concentration was determined by the methods of Lowry et al. (Lowry et al., 1951). BP mutagenesis assay was performed according to the method of Ames et al. (Ames et al., 1975) with modifications. Overnight culture of TA 98 strain of Salmonella typhimurium (0.1 ml) was pre-incubated for 20 min at 37°C with the liver S-9 (40 mg/ml) incubation mixture containing complete NADPH generating system and NADH (S-9 co-factor mix, Oriental East Co., Tokyo) and BP (5 µg) in DMSO and in some experiments, anti-P-450 BTL (P-450 2D subfamily) antiserum prepared against purified P-450 BTL (Suzuki et al., 1992) in a final volume of 0.7 ml. Judging from the western blotting analysis, this antiserum does not cross-react with P-450 proteins of molecular weight other than 50,000 dt, suggesting that it is highly specific to P-450 2D. The pre-incubation mixture without BP in test tubes was put in water bath at 37°C for 5 min for temperature equilibration. The substrate BP was then added to start the reaction. After 20 min pre-incubation, the reaction was halted by placing the reaction tubes in ice cold water, followed by the addition of 2 ml of soft agar containing 0.5 mM L-histidine and 0.5 mM biotin. The mixture was immediately poured on to glucose agar plates. The number of histidine revertant colonies was counted after 2 days of incubation at 37°C. The numbers of spontaneous revertants were subtracted.

Figure 1 indicates the numbers of histidine revertant colonies obtained after pre-incubation of BP with liver S-9 from male and female Wistar and DA rats. Also shown are those after pre-incubation with liver S-9 from rats of both strains pretreated with 3-MC, an inducer of cytochrome P-450 1A1. It is apparent from this figure that 3-MC-treatment induced in both strains of rats high ability to activate BP to a mutagen. We have previously shown (Fujita et al., 1988) that anti P-450 1A1 antibody dramatically inhibited the mutagenic activation of BP by liver S-9 from 3-MC treated rats, but hardly inhibited the activa-

![Fig. 1. Effect of strain, sex and induction of P-450 on the ability of rat liver S-9 to mutagenically activate benzo(a)pyrene. Liver S-9 from male (M) and female (F) rats of Wistar and DA strains (left) as well as 3-methylcholanthrene treated male rats of these strains (right) were used to test their ability to transform benzo(a)pyrene to a mutagen. Ames methods were used to detect the degree of mutagenic transformation elicited by these livers S-9's. Error bars are S.D. from the mean of three determinations.](image-url)
Involvement of P-450 2D in mutagenic activation of benzo(a)pyrene.

Fig. 2. Effect of anti-P-450 BTL (P-450 2D subfamily) antibody on mutagenic transformation of benzo(a)pyrene by liver S-9. Prior to the onset of the pre-incubation, the liver S-9 was mixed with anti-P-450 BTL (2.5, 5, 7.5, 15 and 20 mg/mg S-9) or pre-immune rabbit serum as the control and let stand for 30 min on ice. Then reaction mixture was prepared and prewarmed for 5 min and the pre-incubation was started (see text). The remaining activity in the presence of antibody was shown. Error bars are S.D. from the mean of three determinations.

P-450 2D1 (Suzuki et al., 1992), but did not inhibit imipramine N-demethylase activity (not shown), which is catalyzed by cytochrome P-450 2C11 for the large part (Fujita et al., 1989).

Taken altogether, it can be concluded that although cytochrome P-450 1A1 may play the major role in activating BP in livers from 3-MC-treated rats, the constitutive cytochromes P-450 2D subfamily in addition to possible involvement of P-450 2C11 plays a role in BP activation in livers from untreated rats. Further study is needed to elucidate which form of P-450, induced or constitutive, plays the major role in activation of the carcinogen in actual carcinogenesis.

ACKNOWLEDGMENTS

This work was supported in part by a Grant-in-Aid for scientific research from the Ministry of Education, Science and Culture of Japan to S. F. (No. 04404019)
REFERENCES


