METABOLIC ACTIVATION OF AFLATOXIN B₁ BY HUMAN PLACENTAL MICROSOMES

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ABSTRACT — The metabolic activation of aflatoxin B₁ by human placental microsomes was studied. Aflatoxin B₁ showed relatively high mutagenic activity in Ames test when incubated with human placental microsomes. Addition of α -naphthoflavone or aminogluthethimide, known inhibitors of cytochrome P450 1A and P450 19, respectively, into the test system partially inhibited the mutagen-producing activity. It was suggested that the activation of aflatoxin B₁ in human placental microsomes is mediated by at least these two forms of cytochrome P450.

KEY WORDS : Human placenta, Aflatoxin B₁, Mutation assay

Placenta has been considered as an important organ that is necessary for fetus protection and development. Although fetal and maternal circulatory systems normally never merge, diffusion of substances can occur through the placenta. Thus the studies on transfusion and metabolism of xenobiotics, i. e., drugs or environmental pollutants, by human placenta have priority. Aflatoxin B₁ (AFB₁) is a potent carcinogen produced in *Aspergillus flavus* and causes hepatic carcinomas to most species studied, including non-human primates (Adamson and Sieber, 1983). AFB₁ is also a potent mutagen and teratogen. An embryotoxicity has been demonstrated in many animal species (Heyes, 1981). Recently, Denning et al. (1990) reported the transplacental transfer of aflatoxins in humans, which is of biological importance since placental or post-placental modification of AFB₁ to more

toxic form is possible. Biotransformation of AFB₁ appears to be a major determinant of the potency of its effects. In animal livers, AFB₁ is activated via formation of AFB₁-8,9-epoxide, leading to covalent binding to biological macromolecules (Martin and Garner, 1977). Recently, we purified P450HFLa from human fetal livers (Kitada et al., 1985 ; 1987a ; 1987b), and showed that the P450HFLa was a major form that activates AFB₁ in human fetal liver (Kitada et al., 1990 ; 1991). Despite these studies, we were not aware of the possibility whether placenta are involved in the metabolic activation of AFB₁. In the present study, we found that the normal placenta could activate AFB₁ and that multiple forms of cytochrome P450 may be responsible for the activation. These findings will be of significant importance for assessment of genotoxicity and embryotoxicity of AFB₁.

Placenta from eleven subjects were frozen at –80°C immediately after handling out to us at

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normal delivery and stored until use. A portion of each placenta was minced and homogenized in three volumes of 1.15% KCl. The homogenates were centrifuged at $9,000\times g$ for 20 minutes (4°C) and the supernatants were further centrifuged at $105,000\times g$ for 60 minutes. Resulting microsomal pellet was homogenized in water. Ames test was performed according to the literature (Ames et al., 1975) with minor modifications. Briefly, $100\ \mu\text{l}$ of an overnight cultured *Salmonella typhimurium* TA100 (kindly provided by Dr. Nohmi, National Institute of Hygienic Sciences, Tokyo) was mixed with $250\ \mu\text{l}$ of an NADPH-generating system (Kitada et al., 1987b) and $250\ \mu\text{l}$ of placental microsomes ($250\ \mu\text{g}$ protein). Incubation at 37°C was started by adding $10\ \mu\text{l}$ of AFB_1 (Macor Chemicals, Israel) dissolved in dimethylsulfoxide (DMSO). After being incubated for 20 minutes, the mixture was inoculated to the Vogel-Bonner plate with a soft agar solution. The number of colonies (His^+ phenotype) was counted after incubation for 48 hours at 37°C . As a control experiment, only DMSO was added instead of AFB_1 solution in DMSO.

We first examined the abilities of human placental microsomes to activate AFB_1 at various concentrations (Figure 1). The results clearly demonstrated that AFB_1 was metabolically activated by human placental microsomes, and that the activation was concentration-dependent up to $4\ \mu\text{g}\ \text{AFB}_1/\text{ml}$. The abilities of placental microsomes to activate AFB_1 were not restricted in certain subjects. The results using 11

Fig. 1. Mutagenic activation of AFB_1 by human placental microsomes. *Salmonella typhimurium* TA100 was preincubated in the presence (●) or absence (▲) of human placental microsomes ($250\ \mu\text{g}$ protein) with the indicated concentrations of AFB_1 . After 48 hours post-plating, the number of His^+ -phenotype colonies were counted.

Fig. 2. Inter-individual differences of placenta in the ability of mutagenic activation of AFB_1 . Microsomes from 11 normal placenta were prepared and assayed.

placental samples are shown in Figure 2. The His^+ revertants were induced in all placental samples; the number varies from 496 to 1018 (mean 663 ± 180 revertants/plate at $4\ \mu\text{g}\ \text{AFB}_1/\text{ml}$). The variations in the numbers may be due to inter-individual difference as well as the time prior to freezing. It has been accepted that forms of P450 can activate AFB_1 (Crespi et al., 1991). Thus, the effects of inhibitors for certain forms of P450 on the activation of AFB_1 was examined. Since placenta has relatively high arylhydrocarbon hydroxylase activity that is inducible by cigarette smoking, etc., α -naphthoflavone (Wako Pure Chemicals, Tokyo) was employed to inhibit P450 1A (Huang, 1981). Aminoglutethimide (Sigma, St. Louis, MO, USA) was also used to inhibit P450 19 (Santen et al., 1980), a constitutive form of P450 in human placenta. These inhibitors were added in the preincubation mixture containing *S. typhimurium* TA100 and AFB_1 at an adequate concentration. The results of inhibition assay are shown in Figure 3. Interestingly, both α -naphthoflavone and aminoglutethimide similarly reduced the number of His^+ colonies by about 60% at the highest concentrations of inhibitors. Direct toxicity of the both inhibitors to bacterial cells were monitored by the counting colonies on histidine-containing VB plates inoculated with

Fig. 3. Inhibition of AFB₁ activation mediated by human placental microsomes by α -naphthoflavone and aminoglutethimide. Ames test was carried out in the presence of desired concentrations of α -naphthoflavone (A) and aminoglutethimide (B). The activity (●) was shown as the percentage of the number of revertants of controls assayed in the absence of an inhibitor. The number of revertants in the absence of AFB₁ (66 / plate) was subtracted. Survival rates of bacterial cells were also indicated (×).

bacteria that were cultivated for 20 minutes with corresponding concentrations of inhibitors. Since no direct killing effects were observed with both inhibitors, these inhibitory effects were judged to be completely due to P450-directed inhibition.

We conclude tentatively that human placental microsomes contain forms of P450 that can activate AFB₁ to a ultimate mutagen(s). According to the inhibition assay, we assume that at least two forms of P450, P450 1A and P450 19, are responsible for the AFB₁ activation in human placental microsomes. Denning et al. (1990) reported that sera from cord blood contained higher concentration of AFB₁ than maternal sera. AFB₁ is so lipophilic compound that the transfusion of AFB₁ from cord blood serum to placental villi may occur. Identification and cDNA cloning of a major form(s) of P450 responsible for AFB₁ activation is necessary.

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REFERENCES

- Adamson, R. H. and Sieber, S. M. (1983): Chemical carcinogenesis studies in nonhuman primates. *In* Organ and Species Specificity in Chemical Carcinogenesis (R. Langenbach, et al., eds.) pp. 129–156. Plenum Publishing Corp., New York.
- Ames, B. N., McCann, J. and Yamasaki, E. (1975): Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian microsome mutagenicity test. *Mutation Res.*, **31**, 347–364.
- Crespi, C. L., Penman, B. W., Steimel, D. T., et al. (1991): The development of a human cell line stably expressing human CYP3A4: role in the metabolic activation of aflatoxin B₁ and comparison to CYP1A2 and CYP2A3. *Carcinogenesis*, **12**, 355–359.
- Denning, D. W., Allen, R., Wilkinson, A. P., et al. (1990): Transplacental transfer of aflatoxin in humans. *Carcinogenesis*, **6**, 1033–1035.
- Heyes, A. W. (1981): *Micotoxin Teratogenicity and Mutagenicity*. CRC Press, Boca Raton, LA.
- Huang, M. T., Johnson, E. F., Muller-Eberhard, U., et al. (1981): Specificity in the activation and inhibition of flavonoids of benzo[a]pyrene hydroxylation by cytochrome P-450 isozymes from rabbit liver microsomes. *J. Biol. Chem.*, **256**, 10897–10901.
- Kitada, M., Kamataki, T., Itahashi, K., et al. (1985): Purification and properties of cytochrome P-450 from homogenates of human fetal livers. *Arch.*

- Biochem. Biophys., **241**, 275–280.
- Kitada, M., Kamataki, T., Itahashi, K., et al. (1987a) : Significance of cytochrome P-450 (P-450 HFLa) of human fetal livers in the steroid and drug oxidations. *Biochem. Pharmacol.*, **36**, 453–456.
- Kitada, M., Kamataki, T., Itahashi, K., et al. (1987b) : P-450 HFLa, a form of cytochrome P-450 purified from human fetal livers, is the 16 α -hydroxylase of dehydroepiandrosterone 3-sulfate. *J. Biol. Chem.*, **262**, 13534–13537.
- Kitada, M., Taneda, M., Ohta, K., et al. (1990) : Metabolic activation of aflatoxin B₁ and 2-amino-3-methylimidazo [4, 5-f]-quinoline by human adult and fetal livers. *Cancer Res.*, **50**, 2641–2645.
- Kitada, M., Taneda, M., Itahashi, K., et al. (1991) : Four forms of cytochrome P-450 in human fetal livers : Purification and their capacity to activate promutagens. *Jpn. J. Cancer Res.*, **82**, 426–432.
- Martin, C. N. and Garner, R. C. (1977) : Aflatoxin B₁-oxide generated by chemical or enzymic oxidation of aflatoxin B₁ causes guanine substitution in nucleic acids. *Nature*, **267**, 863–865.
- Santen, R. J., Samojlik, E. and Wells, S. A. (1980) : Resistance of the ovary to blockade of aromatization with aminoglutethimide. *J. Clin. Endocrinol. Metab.*, **51**, 473–477.