Metabolic Activation of Aflatoxin B₁ and 2-Amino-3-methylimidazo[4,5-f]quinoline by Human Adult and Fetal Livers

Mitsukazu Kitada, Masaki Taneda, Kazuhide Ohta, Kazuo Nagashima, Koshiro Itahashi, and Tetsuya Kamataki

Division of Analytical Biochemistry, Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060, Japan, and Department of Pathology, Faculty of Medicine, Hokkaido University [K. N.], Kawasaki Seitetsu Hospital, Chiba 280, Japan [K. I.]

ABSTRACT

The mutagenic activation of promutagens by human adult and fetal livers was investigated using the umu test system. Among the promutagens studied, aflatoxin B₁ (AFB₁) and 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) were efficiently activated to mutagens by both adult and fetal livers. 7,8-Benzoflavone inhibited the activation of IQ by fetal livers, but the inhibition observed in adult livers was much less than that observed in adult livers. Antibodies to P450HM1 (P450IIIA4) and P450HFLa markedly inhibited the activation of AFB₁, by adult and fetal livers, respectively. The formation of genotoxic product(s) from IQ in human adult livers was almost completely inhibited by anti-P4481 (P450IA2) antibodies but not by anti-P450HMI1 antibodies, whereas that in fetal livers was inhibited by both anti-P450HFLa and anti-P450IA2 antibodies. P450HFLa catalyzed the mutagenic activation of both AFB₁, and IQ in a reconstructed system. On the contrary, P450HM1 catalyzed the mutagenic activation of AFB₁, but not IQ. A preparation of cytchrome P450 partially purified from human fetal livers and cross-reactive with anti-P450IA2 antibodies was found to be active for mutagenic activation of IQ in a reconstructed system.

These results indicate that P450HFLa and P450HM1 are mainly involved in the genotoxic product formation from AFB₁ in fetal and adult livers, respectively, and that the metabolic activation of IQ in fetal livers is catalyzed by two forms of cytchrome P450, P450HFLa, and cytochrome P450 immunologically related to P450IA2 but that in adult livers it is mainly catalyzed by cytchrome P450 related to P450IA2.

INTRODUCTION

Cytochrome P450 exhibits distinct but overlapping substrate specificities for the metabolism of a wide variety of structurally unrelated compounds such as endogenous steroids, fatty acids, and exogenous drugs and other chemicals (7). It is not surprising, therefore, that cytochrome P450 metabolizes xenobiotics to form chemically high-reactive metabolites, which may exert mutagenic, carcinogenic, and cytotoxic activities (8).

Human fetal livers are capable of metabolizing xenobiotics at an early gestational age (9). Several lines of evidence have supported the idea that multiple forms of cytchrome P450 are also present in human fetal livers (10, 11). Recently, one of the forms of cytchrome P450 (P450HFLa) has been purified to an electrophoretically homogeneous from human fetal livers (6) and has been suggested to belong to the same gene family as P450NF (P450IIIA4) which was purified from human adult livers. Antibodies to P450HFLa (P450IIIA4) purified from adult livers in their activating capacities of promutagens such as IQ, although P450HFLa and P450HM1 are closely related to each other in their structural and immunochromical properties. In addition, the data presented here indicate that cytochrome P450 immunologically related to P450IA2 is expressed in fetal livers and is active for mutagenic activation of promutagens.

Received 9/13/89; revised 1/4/90.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was partly supported by a Grant-in-Aid for Cancer Research from the Ministry of Education Science and Culture of Japan and the Uehara Memorial Foundation.

2 To whom requests for reprints should be addressed.

3 The abbreviations used are: P450, liver microsomal cytochrome P450. P-448-H is referred to as P450d (1) and P450ISF-G (2). P450HM1 is probably identical with HLP (3), P450ONF (4), and P450 human-1 (5). P450HFLa is one of the forms of cytochrome P450 in human fetal livers (6); Try-P-2, 3-amino-1-methyl-5-fluoropyridine-4,3'-indole acetate; Glu-P-1, 2-amino-6-methylidopyrido(1,2-a:3',2'-d)imidazole acetate; AFB₁, aflatoxin B₁; IQ, 2-amino-3-methylimidazo(4,5-f)quinoline; MeIQ, 2-amino-3,4-dimethylimidazo(4,5-f)quinoline; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

MATERIALS AND METHODS

Purification of Cytochrome P450 from Human Livers. Livers were obtained from fetuses, stillborn due to heart failure and immaturity, and postmortem human adults and were frozen at —70°C until use. Cytochrome P450 cross-reactive with anti-P450IA2 antibodies was purified from homogenates of fetal livers (20 to 23 weeks) as follows. Homogenates solubilized with sodium cholate (0.6%, w/v) were applied to an aminocetyl Sepharose 4B column which had been equilibrated with 100 mM potassium phosphate buffer (pH 7.25) containing 20% glycerol, 1 mM EDTA, 0.1 mM dithiothreitol, 0.6% sodium cholate, and 0.1 mM phenylmethylsulfonyl fluoride. After the column was washed with equilibrating buffer, cytochrome P450 was eluted by washing the column with 100 mM potassium phosphate buffer (pH 7.25) containing 20% glycerol, 1 mM EDTA, 0.1 mM dithiothreitol, 0.1% sodium cholate, and 0.5% Emulgen 911. Fractions containing cytochrome P450 cross-reactive with anti-P450IA2 antibodies were pooled and concentrated by ultrafiltration on a UK-50 membrane (Toyko, Tokyo, Japan). The concentrated fraction was diluted 10-fold with 20 mM Tris-acetate buffer (pH 7.5) containing 20% glycerol and subjected to fast protein liquid chromatography system (Pharmacia) equipped with a preparative DEAE-SPW column (Toso, Tokyo, Japan) which had been equilibrated with 20 mM Tris-acetate buffer (pH 7.5) containing 20% glycerol and 0.4% Emulgen 911.

After the column was thoroughly washed with the equilibrating buffer, the cytochrome P450 adsorbed was eluted by washing the column with a linear gradient of sodium acetate concentration (0 to 300 mM). The molecular weight of the cytochrome P450 was estimated to be about 48,000. Judging from SDS-PAGE, this cytochrome P450 fraction was found to contain small amounts of proteins other than cytochrome P450. Purification of P450HFLa from human fetal livers and P450HM1 from human adult livers was conducted as described elsewhere (6, 13). Final preparations of P450HFLa and P450HM1 were electrophoretically homogeneous on SDS-PAGE.

Assay for the Mutagenic Activation of Promutagens. Liver homogenates of human fetuses and liver microsomes of human adults were prepared as described previously (13, 16). The induction of the umu gene expression by metabolic activation of promutagens was measured using the tester strain Salmonella typhimurium TA1535/pSK1002 carrying the plasmid, pSK1002, which contains a umuC'-lacZ-fused gene that produces a hybrid protein with β-galactosidase activity (17, 18). Since the expression of umuC'-lacZ-fused gene is under the control of the umu gene promoter, the ability of the DNA-damaging agents to induce the umu gene operon can be monitored by measuring the level of β-

2641

Downloaded from cancerres.aacrjournals.org on April 12, 2017. © 1990 American Association for Cancer Research.
Table 1  Effect of 7,8-benzoflavone on mutagenic activation of AFB, and IQ in human fetal and adult livers

<table>
<thead>
<tr>
<th>Addition</th>
<th>Fetus (β-galactosidase units/min/mg)</th>
<th>Adult (β-galactosidase units/min/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AFB1</td>
<td>IQ</td>
</tr>
<tr>
<td>None</td>
<td>4.2 ± 0.1 (100)*</td>
<td>3.5 ± 0.1 (100)</td>
</tr>
<tr>
<td>7,8-Benzoflavone</td>
<td>5.6 ± 0.1 (134)</td>
<td>2.8 ± 0.1 (79)</td>
</tr>
<tr>
<td>100 μM</td>
<td>4.7 ± 0.1 (111)</td>
<td>2.5 ± 0.1 (72)</td>
</tr>
<tr>
<td>200 μM</td>
<td>8.9 ± 0.1 (135)</td>
<td>0.8 ± 0.1 (15)</td>
</tr>
</tbody>
</table>

*Values represent the mean ± SD of duplicate determinations. Numbers in parentheses, percentages.

Among the promutagens studied, AFB1, IQ, and MelIQ were efficiently activated to mutagens by human fetal livers. Other pyrolysate arylamines such as Trp-P-2 and Glu-P-1 were also activated to mutagens but to a lesser extent than IQ and MelIQ. In the case of adult livers, microsomes were used as an enzyme source, whereas in the case of fetal livers, since most cytochrome P450 in homogenates of human fetal livers has been shown to recover in the 200 x g pellet (26), we used whole homogenates as an enzyme source for the assay of mutagenic activation. Therefore, it is reasonable to assume that fetal livers activated these promutagens at rates comparable to or faster than did adult livers.

Fig. 1 shows the effects of 7,8-benzoflavone on metabolic activation of AFB, and IQ in human fetal and adult livers. 7,8-Benzoflavone slightly enhanced the mutagen production from AFB, while it inhibited the mutagenic activation of IQ by both fetal and adult livers. The inhibition of metabolic activation of IQ in adult livers was much greater than that observed in fetal livers.

Fig. 2 shows the effects of antibodies to P450HFLa, P450HM1, and P450IA2 on the activation of AFB1. As expected from the results shown in Table 1, anti-P450IA2 antibodies did not affect the mutagenic activation of AFB1 in either fetal or adult livers. In contrast to anti-P450IA2 antibodies, anti-P450HFLa and anti-P450HM1 antibodies strongly inhibited the activation of AFB1 in fetal and adult livers, respectively. As shown in Fig. 3, both antibodies to P450HFLa and P450IA2 inhibited the metabolic activation of IQ in fetal livers. On the other hand, the genotoxic product formation from IQ in adult livers was almost completely inhibited by anti-P450IA2 antibodies but not by anti-P450HM1 antibodies. These results indicate that P450HFLa is one of the forms of cytochrome P450 involved in the metabolic activation of IQ in human fetal livers, but P450HM1 is not responsible for the activation of IQ in adult livers. The results indicating that anti-P450IA2 antibodies partially inhibited the mutagenic activation of IQ in fetal livers.

RESULTS

As shown in Fig. 1, human fetal livers were capable of activating promutagens such as AFB1, Glu-P-1, IQ, and MelIQ.
livers probably suggest that cytochrome P450 immunochemically related to P450IA2 is also involved in the mutagenic activation of IQ in fetal livers. Thus, to clarify the possibility of whether cytochrome P450 cross-reactive with anti-P450IA2 antibodies catalyzes the mutagenic activation of IQ, we partially purified cytochrome P450 immunochemically related to P450IA2 from human fetal livers. Fig. 4 shows the elution profile of cytochrome P450 from a preparative DEAE-5PW column. The eluate was monitored for cytochrome P450 with absorbance at 405 nm. Each fraction was subjected to immuno blotting with anti-P450IA2 antibodies to examine cross-reactive protein before fractions were combined. Cytochrome P450 immunochemically reactive with anti-P450IA2 antibodies was eluted into the fractions shown by oblique lines. Since the amounts of cytochrome P450 eluted in these fractions after one run of chromatography were insufficient for further purification, the purification was repeated and the eluates were pooled. The pooled fraction was dialyzed against 10 mM potassium phosphate buffer (pH 7.25) containing 20% glycerol and then applied onto a hydroxylapatite column to remove excess Emulgen 911. The results of SDS-PAGE of partially purified cytochrome P450 (tentatively termed P450HFLb) and Western blot analysis with anti-P450IA2 antibodies are shown in Fig. 5. Final preparation of P450HFLb showed cross-reactivity with anti-P450IA2 antibodies but still contained small amounts of proteins other than cytochrome P450. Table 2 shows the metabolic activation of AFBl, IQ, and Glu-P-1 by purified prepa-
In the present study, we investigated the mutagen-producing activity of human fetal livers. As expected from the results reported by Shimada and Guengerich (15), anti-P450HM1 antibodies almost completely inhibited the mutagenic activation of AFB1 in human adult livers. In addition, anti-P450HFLa antibodies strongly inhibited the mutagenic activation of AFB1 in human fetal livers as reported previously (36). Several investigators (15, 37) have shown that the mutagenic activation of AFB1 in adult livers was enhanced by 7,8-benzoflavone. Although the mechanism(s) by which 7,8-benzoflavone stimulated the mutagenic activation were not known, 7,8-benzoflavone also stimulated the mutagenic activation of AFB1 in fetal livers at a concentration of 100 \( \mu \)M, suggesting similar properties of cytochrome P450 involved in the activation in fetal and adult livers.

Anti-P450IA2 antibodies almost completely inhibited the mutagenic activation of IQ in adult livers. The antibodies also inhibited the mutagen-producing activity from IQ in fetal livers but to a lesser extent than seen in adult livers, suggesting that cytochrome P450 other than cytochrome P450 immunochemically related to P450IA2 might also be involved in the mutagenic activation. In view of the results that P450HFLa catalyzed mutagen production from IQ in a reconstituted system and that anti-P450HFLa antibodies inhibited the mutagen production by fetal livers, we propose that P450HFLa is one of the forms of cytochrome P450 responsible for the mutagenic activation of IQ in fetal livers. In addition, the results presented indicate that P450HFLa is somewhat different from P450HM1 (P450IIIA4) in the mutagen(s)-producing activities and cytochrome \( b_5 \) requirement for the activities, although P450HFLa and P450HM1 (P450IIIA4) have been shown to be highly homologous in their structural and immunochemical properties (14).

Anti-P450IA2 antibodies inhibited genotoxic product formation from IQ in fetal livers. Therefore, to clarify the possibility of whether cytochrome P450 cross-reactive with anti-P450IA2 antibodies catalyzes the mutagenic activation of IQ, P450HFLb, which is immunochemically related to P450IA2, was partially purified from fetal livers. P450HFLb was capable of activating IQ to mutagen in a reconstituted monooxygenase system. Adult human cytochrome P450 (P450PA) involved in phenacetin \( O \)-deethylation (38) has been shown to be responsible for the mutagenic activation of IQ in adult livers (39). P450HFLb seemed somewhat different from P450PA reported by Distlerath et al. (39) in molecular weight and purification procedures. It is, furthermore, not known whether P450HFLb is capable of metabolizing phenacetin. Therefore, the similarity of both forms of cytochrome P450 in their physicochemical properties cannot be established at present.

Contrary to our expectations, P450HFLb was also active in mutagenic activation of AFB1 in the presence or absence of cytochrome \( b_5 \). Anti-P450HFLb antibodies did not show any effects on genotoxic product formation from AFB1 in fetal livers, P450HFLb may not contribute to the mutagenic activation in fetal livers. This remains to be clarified using the antibodies to P450HFLb.

**ACKNOWLEDGMENTS**

We wish to express our appreciation to Dr. Minako Nagao, National Cancer Research Institute, Tokyo, for providing some promutagens.

**REFERENCES**

1. Ryan, D. E., Thomas, P. E., and Levin, W. Hepatic microsomal cytochrome P-450 from rats treated with isosafrole: purification and characterization of...
Metabolic Activation of Aflatoxin B₁ and 2-Amino-3-methylimidazo[4,5-f]-quinoline by Human Adult and Fetal Livers

Mitsukazu Kitada, Masaki Taneda, Kazuhide Ohta, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/50/9/2641

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.