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CYP2A13 expressed in human bladder metabolically activates 4-aminobiphenyl

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Abstract

Cigarette smoking is the predominant risk factor for bladder cancer. Aromatic amines such as 4-aminobiphenyl (ABP) are the major carcinogens found in tobacco smoke. Although it is generally accepted that ABP is metabolically activated via $N$-hydroxylation by CYP1A2 in human liver, previous studies using Cyp1a2-null mice indicated the involvement of other enzyme(s). Here we found that CYP2A13 can metabolically activate ABP to show genotoxicity by *Umu* assay. The Km and Vmax values for ABP $N$-hydroxylation by recombinant CYP2A13 in *E. coli* were 38.5 ± 0.6 µM and 7.8 ± 0.0 pmol/min/pmol CYP, respectively. The Km and Vmax values by recombinant CYP1A2 were 9.9 ± 0.9 µM and 39.6 ± 0.9 pmol/min/pmol CYP, respectively, showing 20-fold higher intrinsic clearance than CYP2A13. In human bladder, CYP2A13 mRNA, but not CYP1A2, is expressed at a relatively high level. Human bladder microsomes showed ABP $N$-hydroxylase activity (Km = 34.9 ± 4.7 µM and Vmax = 57.5 ± 1.9 pmol/min/mg protein), although the intrinsic clearance was 5-fold lower than that in human liver microsomes (Km = 33.2 ± 2.0 µM and Vmax = 293.9 ± 5.8 pmol/min/mg protein). The activity in human bladder microsomes was prominently inhibited by 8-methoxypsoralen, but not by fluvoxamine, anti-CYP1A2 or anti-CYP2A6 antibodies. CYP2S1, which is expressed in human bladder and has relatively high amino acid identities with CYP2As, did not show detectable ABP $N$-hydroxylase activity. In conclusion, although the enzyme responsible for ABP $N$-hydroxylation in human bladder microsomes could not be determined, we found that CYP2A13 metabolically activates ABP.

**Key words:** cytochrome P450; 4-aminobiphenyl; metabolic activation; bladder cancer
Introduction

The human CYP2A gene subfamily has two functional genes, CYP2A6 and CYP2A13. CYP2A6 is predominantly expressed in liver, whereas CYP2A13 is predominantly expressed in the respiratory tract. Both enzymes are involved in the metabolism of nicotine and metabolic activation of tobacco-specific nitrosamines such as 4-(methylnitrosamo)-1-(3-pyridyl)-1-butane (NNK). In vitro studies using recombinant CYPs revealed that CYP2A13 is much more active than CYP2A6 in the metabolism of these substrates.

4-Aminobiphenyl (ABP) is a major component of tobacco smoke and is recognized as a human bladder carcinogen. ABP is metabolized to N-hydroxy-4-aminobiphenyl (N-OH-ABP) mainly in liver, and this is a precursor to the formation of ABP-DNA adducts in liver and bladder. Numerous studies indicated that the enzyme primarily responsible for the N-hydroxylation of ABP is CYP1A2. However, studies using Cyp1a2-null mice by two research groups demonstrated that the incidences of hepatocellular carcinoma and ABP-DNA adduct formation were not affected by knockout of Cyp1a2, indicating that CYP1A2 is not the sole CYP required for the metabolic activation of ABP. In the present study, we found that CYP2A13 is highly expressed in bladder as well as lung. We investigated whether ABP can be N-hydroxylated by human bladder microsomes or recombinant CYP2A13.

Materials and methods

Chemicals and reagents

ABP, tranylcypromine hydrochloride, and 8-methoxypsoralen were from Sigma-Aldrich (St. Louis, MO). N-OH-ABP was previously synthesized (6). Coumarin and α-naphthoflavone were purchased from Wako Pure Chemical Industries (Osaka, Japan). Furafylline and fluvoxamine maleate were from Funakoshi (Tokyo, Japan) and Tocris Cookson (Ballwin, MO), respectively. NADP+, glucose-6-phosphate, and
glucose-6-phosphate dehydrogenase were purchased from Oriental Yeast (Tokyo, Japan). ReverTra Ace was purchased from TOYOBO (Osaka, Japan). Taq polymerase was purchased from Greiner Japan (Tokyo, Japan). Random hexamer and Takara Ex Taq R-PCR Version 1.0 were from Takara (Shiga, Japan). Monoclonal antibodies against human CYP1A2 or CYP2A6 were obtained from BD Gentest (Woburn, MA). All primers were commercially synthesized at Hokkaido System Sciences (Sapporo, Japan). Other chemicals were of the highest grade commercially available.

**Total RNA from human tissues and RT-PCR analyses**

Total RNA samples from normal human liver (single donor), colon (pooled, n = 2), bladder (pooled, n = 2), breast (pooled, n = 2), ovary (single donor), and uterus (pooled, n = 3) were obtained from Stratagene (La Jolla, CA). Total RNA samples from human normal lung (single donor) and kidney (single donor) were from Cell Applications (San Diego, CA). Total RNA samples from human normal stomach (single donor), adrenal grand (pooled donor, n = 62), and testis (pooled donor, n = 45) were from Clontech (Palo Alto, CA). Total RNA (4 µg) was added to a reaction mixture containing 150 ng of random hexamer, 100 units of ReverTra Ace, 1 x reaction buffer, and 0.5 mM dNTPs in final volume of 40 µl. The reaction mixture was pre-incubated at 30°C for 10 min, incubated at 42°C for 60 min, and heated at 95°C for 10 min to inactivate the enzyme.

PCR reactions were carried out as follows: An 1 µl portion of the reverse transcribed mixture was added to a PCR mixture containing 0.4 µM of each primer, 0.2 mM dNTPs, 1.5 mM MgCl₂, 1.0 U Taq polymerase, and 1 x PCR buffer [67 mM Tris-HCl (pH 8.8), 16.6 mM (NH₄)₂SO₄, 0.45% Triton X-100, and 0.02% gelatin] in a final volume of 25 µl. The primers used for PCR are shown in Table 1. The PCR conditions were as follows: after an initial denaturation at 94°C for 2 min, the amplification was performed by denaturation at 94°C for 25 sec, annealed at 58°C (CYP2A6 and CYP2A7), 60°C (CYP2A13), or 54°C (CYP2S1, CYP1A2, and glyceraldehyde-3-phosphate dehydrogenase, GAPDH) for 25 sec, and extended at 72°C for 30 sec and cycled immediately for 25-35 cycles. PCR products (15 µl) were
analyzed by electrophoresis with 2% agarose gel.

For quantitative analyses, real-time RT-PCR was performed for CYP2 mRNAs. The primers were the same as above. An 1 µl portion of the reverse transcribed mixture was added to a PCR mixture containing 0.4 µM each primer, 0.33 x SYBR Green I, 0.3 mM dNTPs, 3 mM MgCl₂, 1.25 U Ex-Taq HS, and 1 x R-PCR buffer in a final volume of 25 µl. PCR was performed using the Smart Cycler (Cepheid, Sunnyvale, CA) with Smart Cycler Software (Version 1.2b). The PCR conditions were as follows: after an initial denaturation at 95°C for 30 sec, the amplification was performed by denaturation at 94°C for 4 sec, annealing and extension at 64°C (CYP2A6, CYP2A7, and GAPDH), 68°C (CYP2A13), or 62°C (CYP2S1) for 20 sec for 45 cycles. The copy number was calculated with a standard curve that was made with real-time RT-PCR using copy number-quantified PCR product as a template.

**Enzyme preparations**

Pooled human liver microsomes (lot #28) and microsomes from baculovirus-infected insect cells expressing CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4, and CYP3A5 were obtained from BD Gentest. All enzymes were co-expressed with NADPH-cytochrome P450 oxidoreductase (NPR). CYP2A6, CYP2B6, CYP2Cs, CYP2E1, or CYP3A4 were also co-expressed with cytochrome b₅.

Microsomes from baculovirus-infected insect cells expressing CYP2S1 and purified rat liver NPR were kindly provided from Dr. Jun-Yan Hong (University of Medicine and Dentistry of New Jersey, Piscataway, NJ). Microsomes from human bladder cancer were previously prepared. ¹³ *E. coli* membranes expressing CYP1A1/NPR, ¹⁴ CYP1A2/NPR, ¹⁴ CYP2A6/NPR, ¹⁵ and CYP2A13/NPR ¹⁶ were prepared as described previously. The CYP content and NADPH-cytochrome c reductase activity were determined as described previously. ¹⁷-¹⁹

**ABP N-hydroxylase activity**

The ABP N-hydroxylase activity was determined as described previously ⁶ with slight modifications. A typical incubation mixture (0.2 ml total volume) contained 100 mM
potassium phosphate buffer (pH 7.4), an NADPH-generating system (0.5 mM NADP$^+$, 5 mM glucose-6-phosphate, 5 mM MgCl$_2$, 1 U/ml glucose-6-phosphate dehydrogenase), 10 - 200 $\mu$M 4-ABP and 0.5 mg/ml microsomal protein from human livers and bladders or 25 pmol/ml recombinant CYPs. When the recombinant CYP2S1 (10 pmol) was used, the purified rat liver NPR (30 units) was included according to the method by Wang et al.$^{20}$ ABP was dissolved in dimethyl sulfoxide (DMSO) and the final concentration of DMSO in the incubation mixture was 1%. The reaction was initiated by the addition of the NADPH-generating system following a 1-min pre-incubation at 37°C. The reaction mixture was incubated for 5 min and was terminated by the addition of 0.1 ml of ice-cold acetonitrile. Preliminary experiments indicated that ABP $N$-hydroxylase activity was linear with an incubation time of at least 10 min. The mixture was centrifuged at 4,000 g for 5 min, and the supernatant was immediately subjected to HPLC.

The HPLC equipment consisted of an L-7100 pump (Hitachi, Tokyo, Japan), an L-7200 autosampler (Hitachi), an L-7300 column oven (Hitachi), an L-7500 integrator (Hitachi) with a MightySil RP-18 (150 x 4.6 mm; 5 $\mu$m) column (Kanto Chemical, Tokyo, Japan). The eluent was monitored at 254 nm using an L-7400 UV detector (Hitachi). The mobile phase was 60% acetonitrile containing 0.1% acetic acid. The flow rate was 0.5 ml/min and the column temperature was 35°C. Retention times of $N$-OH-ABP and ABP were 6.4 min and 7.6 min, respectively. The quantification of the metabolite was performed by comparing the HPLC peak height to that of an authentic standard.

Kinetic parameters were estimated from the fitted curves using a computer program (KaleidaGraph, Synergy Software, Reading, PA) designed for nonlinear regression analysis. Michaels-Menten equation, $v = V_{max} \cdot \frac{S}{(K_m + S)}$, was applied.

**Inhibition analysis of ABP $N$-hydroxylase activity**

Chemical inhibitors of CYP1A2 or CYP2A6 were investigated for their effects on the ABP $N$-hydroxylase activities in human liver and bladder microsomes, recombinant CYP1A2 or CYP2A13 expressed in *E. coli* at a substrate concentration of 100 $\mu$M. Fluvoxamine,$^{21}$
furafylline, and α-napthoflavone are inhibitors of CYP1A2. 8-Methoxypsoralen is an inhibitor of CYP2A6 and CYP2A13 as well as CYP1A2. Tranylcypromine is a strong inhibitor of CYP2A6 and a weak inhibitor of CYP1A2. Coumarin is an inhibitor of CYP2A6. The concentration of inhibitors ranged from 0.1 to 100 µM. Fluvoxamine, tranylcypromine, and coumarin were dissolved in distilled water. Furafylline, α-napthoflavone, and 8-Methoxypsoralen were dissolved in DMSO such that the final concentration of DMSO in the incubation mixture was 2%. The incubation mixture including chemical inhibitors was pre-incubated for 1 min before the reaction was initiated by the addition of an NADPH-generating system. It was confirmed that the inhibitory effects of 2% DMSO on ABP N-hydroxylase activity were trivial, and the control activity was determined in the presence of 2% DMSO.

The inhibitory effects of monoclonal antibodies against human CYP1A2 or CYP2A6 were evaluated by pre-incubating microsomes with antibody (10 µg/pmol CYP or 0.2 mg/mg protein) on ice for 30 min, followed by the addition of the other incubation component. The ABP N-hydroxylase activity was determined as described above.

Umum assay

The genotoxicities of 4-aminobiphenyl were determined by measuring the induction of umu gene expression in a Salmonella tester strain TA1535/pSK1002 as described previously. Standard incubation mixtures (final volume of 1.0 ml) consisted of E. coli membranes expressing human CYP/NPR with various concentrations of 4-aminobiphenyl in 0.25 ml of 200 mM potassium phosphate buffer (pH 7.4) containing an NADPH-generating system and 0.75 ml of bacterial suspension. For inactivation of the enzyme activities, heat treatment (100°C, 2 min) of the tubes (before adding test chemicals and the bacteria) was carried out. Incubations were carried out at 37°C for 2 hrs and terminated by cooling the mixtures on ice. The induction of umu gene expression by 4-ABP is presented as umu units of β-galactosidase activity/min/nmol CYP.
Results

Expression of CYP mRNA in human normal tissues

The expression levels of CYP2A mRNA were determined by RT-PCR analyses. As shown in Fig. 1A, CYP2A6 and CYP2A7 were expressed in all tissues investigated in this study. In contrast, CYP2A13 mRNA was expressed in liver, lung, bladder, breast, uterus, ovary, and testis. In this study, we first determined the copy numbers of CYP2A mRNA in human tissues by real-time RT-PCR analyses (Figs. 1B-1D). The copy number of hepatic CYP2A6 mRNA was 50-fold and 10,000-fold higher than CYP2A7 and CYP2A13 mRNAs, respectively. The CYP2A6 mRNA is also highly expressed in breast, and moderately expressed in lung, adrenal grand, ovary, and testis. The CYP2A7 mRNA was moderately expressed in lung, breast, and ovary. In contrast, the CYP2A13 mRNA was highly expressed in lung followed by bladder.

For comparison, the expression levels of CYP1A2 and CYP2S1 mRNA were determined by RT-PCR (Fig. 1A). The CYP1A2 mRNA was detected only in liver. CYP2S1 was expressed in all tissues, and its expression level was high in colon, stomach, and bladder (Fig. 1E).

ABP N-hydroxylase activity by recombinant CYPs

The ABP N-hydroxylase activity by recombinant CYPs was determined at a concentration of 100 µM of ABP (Fig. 2). Among the recombinant CYPs in baculovirus-infected insect cells, CYP1A2 showed the highest ABP N-hydroxylase activities (38.8 pmol/min/pmol CYP), and CYP1A1, CYP1B1, CYP2E1, and CYP3A5 showed negligible activity. In E. coli membrane expressing CYPs, CYP1A2 (9.1 pmol/min/pmol CYP) and CYP2A13 (5.6 pmol/min/pmol CYP) showed ABP N-hydroxylase activity.
**Genotoxic activation of ABP by recombinant CYPs**

To determine whether CYPs could mediate the bioactivation of ABP, the *umu* gene expression was investigated with several CYPs expressed in *E. coli* membrane (Fig. 3). The *umu* gene expression by CYP1A2 was increased in an ABP-concentration dependent manner. The activation of ABP was also observed with the recombinant CYP2A13, but not with CYP1A1 and CYP2A6.

**Kinetic analyses of ABP N-hydroxylase activity by recombinant CYPs and human liver or bladder microsomes**

The kinetics of the ABP N-hydroxylase activity by *E. coli* membrane expressing CYP1A2 or CYP2A13 was followed by the Michaelis-Menten equation (Fig. 4A). For CYP1A2, the Km and Vmax values were $9.9 \pm 0.9 \mu M$ and $39.6 \pm 0.9 \text{ pmol/min/pmol CYP}$, respectively, resulting in an intrinsic clearance of $4.0 \mu l/min/pmol CYP$. For CYP2A13, the Km and Vmax values were $38.5 \pm 0.6 \mu M$ and $7.8 \pm 0.0 \text{ pmol/min/pmol CYP}$, respectively, resulting in an intrinsic clearance of $0.2 \mu l/min/pmol CYP$.

The kinetics of ABP N-hydroxylase activity by human liver or bladder microsomes was also followed by the Michaelis-Menten equation (Fig. 4B). The Km values were similar between human liver microsomes ($33.2 \pm 2.0 \mu M$) and human bladder microsomes ($34.9 \pm 4.7 \mu M$), but the Vmax value was higher in human liver microsomes ($293.9 \pm 5.8 \text{ pmol/min/mg protein}$) than in human bladder microsomes ($57.5 \pm 1.9 \text{ pmol/min/mg protein}$). The intrinsic clearance values in human liver and bladder microsomes were $8.9 \mu l/min/mg protein$ and $1.6 \mu l/min/mg protein$, respectively.

**Effects of chemical inhibitors and antibodies of CYP1A2 or CYP2A on ABP N-hydroxylase activity**

The inhibitory effects of the chemical inhibitors on the ABP N-hydroxylase activities by recombinant CYP1A2 (Fig. 5A) and CYP2A13 (Fig. 5B) expressed in *E. coli* were determined. The ABP N-hydroxylase activity by recombinant CYP1A2 was potently inhibited
by α-naphthoflavone (IC50 = 0.08 µM), fluvoxamine (IC50 = 0.6 µM), and 8-methoxypsoralen (IC50 = 1.2 µM). Furafylline showed a weak inhibition (IC50 = 75.1 µM), but tranylcypromine and coumarin did not inhibit the activity. The ABP N-hydroxylase activity by recombinant CYP2A13 was potently inhibited by 8-methoxypsoralen (IC50 = 0.07 µM), and was moderately inhibited by α-naphthoflavone (IC50 = 5.8 µM), coumarin (IC50 = 6.2 µM), and tranylcypromine (IC50 = 8.0 µM). Fluvoxamine showed a weak inhibition (IC50 = 80.0 µM), but furafylline did not inhibit the activity.

The inhibitory effects of the chemical inhibitors on the ABP N-hydroxylase activities by human liver (Fig. 5C) and bladder (Fig. 5D) microsomes were determined. The ABP N-hydroxylase activity by human liver microsomes was potently inhibited by α-naphthoflavone (IC50 = 0.5 µM), 8-methoxypsoralen (IC50 = 0.7 µM), and fluvoxamine (IC50 = 1.0 µM). Furafylline showed a weak inhibition (IC50 = 37.3 µM), but tranylcypromine and coumarin only slightly inhibited the activity (IC50 > 100 µM). The ABP N-hydroxylase activity by human bladder microsomes was potently inhibited by 8-methoxypsoralen (IC50 = 0.5 µM), but not by the other inhibitors.

The inhibitory effects of the monoclonal anti-human CYP1A2 or CYP2A6 antibodies on the ABP N-hydroxylase activities by recombinant CYP1A2 and CYP2A13 expressed in E. coli, and human liver and bladder microsomes were determined (Fig. 6). The activity by recombinant CYP1A2 was inhibited by anti-CYP1A2 antibody (28% of control), but not by anti-CYP2A6 antibody (100% of control). The activity by recombinant CYP2A13 was inhibited by anti-CYP2A6 antibody (13% of control), but not by anti-CYP1A2 antibody (86% of control). The activity by human liver microsomes was inhibited by anti-CYP1A2 antibody (31% of control), but not by anti-CYP2A6 antibody (91% of control). However, the activity by human bladder microsomes was not inhibited by either antibody (107% and 97% of control).
Discussion

In human liver, the CYP2A6 mRNA was more abundant than the CYP2A7 and CYP2A13 mRNA, consistent with previous studies. The interesting fact is that CYP2A6 mRNA is highly expressed in breast. Bièche et al. have reported that the CYP2A6 mRNA level is significantly higher in estrogen receptor α positive breast tumors than in normal breast tissue. CYP2A6 may possibly have a physiological role in breast in relation to estrogens. Although CYP2A7 has been reported to be a non-functional enzyme, CYP2A7 mRNA is highly detected in liver. Previously, it has been reported that CYP2A13 mRNA is detected in human brain, mammary gland, prostate, testis, uterus, and nasal mucosa, but not in heart, kidney, bone marrow, colon, small intestine, spleen, stomach, thymus, or skeletal muscle. Our data were in accordance with the previous studies, but the new finding was that CYP2A13 is relatively highly expressed in bladder.

To investigate whether CYP2A13 can catalyze the metabolic activation of ABP, we used recombinant CYP2A13 expressed in E. coli (Fig. 2). CYP2A13 showed a prominent metabolic activation of ABP, although it was lower than recombinant CYP1A2 expressed in E. coli (5.6 vs 9.1 pmol/min/pmol CYP). Recombinant CYP1A2 in baculovirus-infected insect cells showed higher ABP N-hydroxylase activity (38.8 pmol/min/pmol CYP) than recombinant CYP1A2 expressed in E. coli. The recombinant CYP1A2 in baculovirus-infected insect cells usually shows higher enzymatic activity than other recombinant systems. In order to exclude host factors, we used the recombinant CYP1A2 and CYP2A13 in E. coli in further analyses. We found that the intrinsic clearance of ABP N-hydroxylation by recombinant CYP2A13 was approximately one fifth of that of recombinant CYP1A2 (Fig. 4A).

It has been generally accepted that ABP is metabolically activated by N-hydroxylation by CYP1A2 in human liver, followed by O-acetylation to yield a reactive metabolite, N-acetoxy esters. In accordance with a previous study, we confirmed that CYP1A2 could metabolically activate ABP (Fig. 3). In addition, we found that CYP2A13 also showed the
genotoxicity of ABP. The accumulation of biologically active metabolites of ABP is dependent on the balance between activation and detoxification. ABP and N-OH-ABP might be inactivated by N-acetyltransferase 2 (NAT2) in liver, whereas NAT1 is involved in a major bioactivation step, converting urinary N-OH-ABP to reactive N-acetoxy esters that form covalent DNA adducts. NAT1 is detected in many extrahepatic tissues and is higher in bladder mucosa than NAT2. The new finding of the present study is that ABP can be N-hydroxylated in human bladder microsomes. Thus, if ABP circulates and comes to the bladder, it can be hydroxylated and immediately converted to N-acetoxy esters by NAT1.

To determine whether the CYP isoform involved in ABP N-hydroxylation in human bladder microsomes is CYP2A13, inhibition studies were performed. The inhibition studies for human liver microsomes and recombinant CYP1A2 supported that the CYP isoform responsible for ABP N-hydroxylation in human liver microsomes was mainly CYP1A2. 8-Methoxypsoralen is known to inhibit both CYP2A13 and CYP1A2. However, since CYP1A2 is not expressed in human bladder, the inhibition by 8-methoxypsoralen could suggest the involvement of CYP2A13 in ABP N-hydroxylation in human bladder microsomes. In contrast, the inhibition study using antibodies did not support this notion. Taking these results into consideration, certain CYP isoform(s) other than CYP2A13, which can be inhibited by 8-methoxypsoralen, may also contribute to ABP N-hydroxylation in human bladder microsomes.

CYP2S1 is a newly identified isoform localized on chromosome 19q13.2 close to the CYP2 cluster including CYP2A6 and CYP2A13. The amino acid identities between CYP2S1 and CYP2A are relatively high (48 - 49%). Rylander et al. reported that CYP2S1 is predominantly expressed in human extrahepatic tissues including trachea and lung as well as kidney, stomach, small intestine, colon, and spleen. In addition to these tissues, we found that CYP2S1 is also expressed in bladder, in accordance with a previous study by Saarikoski et al., and its copy number is higher than that of CYP2A13. The known substrates of CYP2S1 are all-trans retinoic acid, naphthalene, and aflatoxin B1. The high level of CYP2S1 expression in human respiratory tissues suggests that this enzyme may have a role in
the metabolism of environmental chemicals. This background prompted us to investigate whether CYP2S1 can catalyze ABP N-hydroxylation and be a contributor in human bladder microsomes. However, recombinant CYP2S1 did not show detectable ABP N-hydroxylase activity. Recently, it has been reported that CYP2S1 can not catalyze the metabolism of NNK and nicotine. Thus, the substrate specificity of CYP2S1 might be different from those of CYP2A6 and CYP2A13.

In conclusion, we found that human bladder microsomes can active ABP N-hydroxylation, although the CYP isoform(s) responsible for the activation could not be identified. The new finding in this study is that CYP2A13, which is expressed in human bladder, metabolically activates ABP.

**Acknowledgements**

We are grateful to Dr. Jun-Yan Hong, University of Medicine and Dentistry of New Jersey (Piscataway, NJ) for providing microsomes from baculovirus-infected insect cells expressing CYP2S1 and purified rat liver NPR. We acknowledge Mr. Brent Bell for reviewing the manuscript.
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Figure legends

**Figure 1.** Expression levels of CYP2A mRNA in various human tissues. (A) Amplification of CYP2A6 (336 bp), CYP2A7 (336 bp), CYP2A13 (301 bp), CYP2S1 (340 bp), CYP1A2 (262 bp), and GAPDH (300 bp) in human tissues by RT-PCR are shown. Relative copy numbers to GAPDH of CYP2A6 (B), CYP2A7 (C), CYP2A13 (D), and CYP2S1 (E) in human tissues were determined by real-time RT-PCR analyses. Each column represents the mean of duplicate determinations.

**Figure 2.** ABP \(N\)-hydroxylase activity by recombinant human CYPs. Microsomes from baculovirus-infected insect cells (A) or *E. coli* membrane (B) expressing each human CYP were incubated with 100 \(\mu\)M of ABP. Each column represents the mean of duplicate determinations.

**Figure 3.** Metabolic activation of ABP by CYP1A2 or CYP2A13 in *S. typhimurium* TA1535/pSK1002. The *umu* gene expression was measured in the absence and presence of the enzyme system for subtracting the direct activities. Each data point represents the mean of duplicate determinations.

**Figure 4.** Kinetic analyses of ABP \(N\)-hydroxylase activity. ABP \(N\)-hydroxylase activities by recombinant CYP1A2 or CYP2A13 in *E. coli* (A) and human liver or bladder microsomes (B) were determined. Each data point represents the mean of duplicate determinations.

**Figure 5.** Effects of CYP inhibitors on ABP \(N\)-hydroxylase activity. ABP \(N\)-hydroxylase activities by recombinant CYP1A2 (A), recombinant CYP2A13 (B) in *E. coli*, human liver (C) or bladder (D) microsomes were determined at a substrate concentration of 50 \(\mu\)M. Fluvoxamine, furafylline, \(\alpha\)-naphthoflavone (CYP1A2), 8-methoxypsoralen (CYP1A2, CYP2A6, and CYP2A13), tranylcypromine, and coumarin (CYP2A6) were used. Each data
point represents the mean of duplicate determinations.

**Figure 6.** Effects of monoclonal antibodies against CYP1A2 or CYP2A6 on ABP $N$-hydroxylase activity. Recombinant CYP1A2 or CYP2A13 in *E. coli* (10 µg/pmol CYP), human liver or bladder microsomes (0.2 mg/mg protein) was pre-incubated with antibodies on ice for 30 min. The ABP $N$-hydroxylase activity was determined at a substrate concentration of 50 µM. Each column represents the mean of duplicate determinations.
Fig. 1. Nakajima et al.
Fig. 2. Nakajima et al.

A) Baculovirus-infected insect cells

B) E. coli

ABP N-hydroxylation (pmol/min/pmol CYP)
Fig. 3. Nakajima et al.
Fig. 4. Nakajima et al.

(A) CYP1A2

Km = 9.9 ± 0.9 µM  
Vmax = 39.6 ± 0.9 pmol/min/pmol CYP

CYP2A13

Km = 38.5 ± 0.6 µM  
Vmax = 7.8 ± 0.0 pmol/min/pmol CYP

(B) ABP N-hydroxylation (pmol/min/mg protein)

human liver microsomes

Km = 33.2 ± 2.0 µM  
Vmax = 293.9 ± 5.8 pmol/min/mg protein

human bladder microsomes

Km = 34.9 ± 4.7 µM  
Vmax = 57.5 ± 1.9 pmol/min/mg protein
Fig. 5. Nakajima et al.

A. Recombinant CYP1A2
B. Recombinant CYP2A13
C. Human liver microsomes
D. Human bladder microsomes

Concentration (µM)

Residual activity (% of control)

- Fluvoxamine
- Furafylline
- α-Naphthoflavone
- 8-Methoxypsoralen
- Tranylcypromine
- Coumarin
Fig. 6. Nakajima et al.

![Graph showing residual activity (%) of control for different samples treated with Anti-CYP1A2 and Anti-CYP2A6 antibodies.](image)