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CYP1A1 and CYP1A2 expression: Comparing ‘humanized’ mouse lines and wild-type mice; comparing human and mouse hepatoma-derived cell lines

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Abstract

Human and rodent cytochrome P450 (CYP) enzymes sometimes exhibit striking species-specific differences in substrate preference and rate of metabolism. Human risk assessment of CYP substrates might therefore best be evaluated in the intact mouse by replacing mouse *Cyp* genes with human CYP orthologs; however, how “human-like” can human gene expression be expected in mouse tissues? Previously a bacterial-artificial-chromosome-transgenic mouse, carrying the human *CYP1A1_CYP1A2* locus and lacking the mouse *Cyp1a1* and *Cyp1a2* orthologs, was shown to express robustly human dioxin-inducible CYP1A1 and basal versus inducible CYP1A2 (mRNAs, proteins, enzyme activities) in each of nine mouse tissues examined. Chimeric mice carrying humanized liver have also been generated, by transplanting human hepatocytes into a urokinase-type plasminogen activator(+/+)_severe-combined-immunodeficiency (*uPA/SCID*) line with most of its mouse hepatocytes ablated. Herein we compare basal and dioxin-induced CYP1A mRNA copy numbers, protein levels, and four enzymes (benzo[*a*]pyrene hydroxylase, ethoxyresorufin *O*-deethylase, acetanilide 4-hydroxylase, methoxyresorufin *O*-demethylase) in liver of these two humanized mouse lines versus wild-type mice; we also compare these same parameters in mouse Hepa-1c1c7 and human HepG2 hepatoma-derived established cell lines. Most strikingly, mouse liver CYP1A1-specific enzyme activities are between 38- and 170-fold higher than human CYP1A1-specific enzyme activities (per unit of mRNA), whereas mouse versus human CYP1A2 enzyme activities (per unit of mRNA) are within 2.5-fold of one another. Moreover, both the mouse and human hepatoma cell lines exhibit striking differences in CYP1A mRNA levels and enzyme activities. These findings are relevant to risk assessment involving human CYP1A1 and CYP1A2 substrates, when administered to mice as environmental toxicants or drugs.

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Note added in proof

A recent study (Wilson et al., 2008) is directly relevant to the problems addressed in our present manuscript. This study involves Tc1 hepatocytes, derived from an aneuploid mouse strain carrying human chromosome (Chr) 21 in addition to the entire mouse genome. The authors compared the regulation of human genes in Tc1 cells to that of the mouse orthologous genes in these same cells, using mouse wild-type versus human wild-type cells as controls. Regulation in the nuclei of Tc1 cells was compared at three levels: binding of transcription factors to DNA, modification of histones, and gene expression. The binding patterns of HNF1 α , HNF4 α and HNF6 on human Chr 21 in Tc1 cells matched closely those seen in human wild-type cells, rather than those seen in mouse wild-type cells. Similarly, histone modifications—as well as gene expression (the amount of mRNA transcribed)—showed human-specific, instead of mouse-specific, patterns on human Chr 21 in Tc1 cells. The authors concluded that it is the regulatory DNA sequence, rather than any other species-specific factor, which is the single most important determinant of gene expression (Wilson et al., 2008).

Keywords

Cytochrome P450 1 (*CYP1*) genes; Bacterial artificial chromosome (BAC); *hCYP1A1_1A2_Cyp1a1/1a2*(-/-) BAC-transgenic mouse line; *uPA/SCID* chimeric mouse line carrying human hepatocytes; Human risk assessment; Western immunoblot; Benzo[*a*]pyrene hydroxylase, ethoxyresorufin *O*-deethylase, acetanilide 4-hydroxylase and methoxyresorufin *O*-demethylase as CYP1A1 and CYP1A2 substrates; 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD, dioxin) as P450 inducer; Mouse Hepa-1c1c7 cell culture; Human HepG2 cell culture

Introduction

The human and mouse genomes comprise 57 and 102 protein-coding cytochrome P450 (**CYP**) genes, respectively, each divided into 18 families (Nelson et al., 2004; Nebert et al., 2004; Nebert and Dalton, 2006). The mammalian *CYP1* gene family encodes three enzymes in both human and mouse—CYP1A1, CYP1A2 and CYP1B1. While the *CYP1A* and *CYP1B* subfamily ancestors diverged from one another ~480 million years ago, *CYP1A2* arose as a duplication event from the *CYP1A1* gene about 420 million years ago. Thus, land animals (including birds) carry both *CYP1A1* and *CYP1A2*; on the other hand, fish genomes do not contain the *CYP1A2* gene (Nelson et al., 1996).

It was originally noted that alteration of a single amino-acid in a CYP protein could change dramatically its catalytic activity from coumarin to testosterone hydroxylation (Lindberg and Negishi, 1989). Similarly, numerous other examples have shown that human and rodent CYP1A2 orthologs, having important amino-acid differences, can display striking species-specific variability in the rates by which certain substrates are metabolized (Turesky, 2005). For example, human and mouse CYP1A2 differ by 3- to 7- fold in catalyzing ethoxyresorufin *O*-deethylation (Aoyama et al., 1989) and uroporphyrinogen oxidation (Nichols et al., 2003).

It therefore can be difficult to extrapolate toxicity or cancer data from rodent studies to human risk assessment. For this reason, we and others have generated “humanized” *hCYP1A1_1A2* transgenic lines in which either mouse *Cyp1a1* or *Cyp1a2* (Jiang et al., 2005; Cheung et al., 2005; Derkenne et al., 2005) or both mouse *Cyp1a1* and *Cyp1a2* (Dragin et al., 2007; Shi et al., 2008) orthologs are ablated. In addition, a global approach for making a humanized mouse has been developed by transplanting human hepatocytes into the urokinase-type plasminogen activator(+/-)_severe-combined- immunodeficiency (*uPA/SCID*) mouse, which otherwise is immunodeficient and undergoes liver failure; these chimeric mice no longer develop liver failure, but rather the mouse liver comprises >70% human hepatocytes that propagate successfully and retain normal pharmacological functions such as drug metabolism (Tateno et al., 2004; Katoh et al., 2008). Eight human P450s (CYP1A2, 2A6, 2C8, 2C9, 2C19, 2D6, 3A4, 3A5), 35 other Phase I enzymes, and four classes of Phase II conjugating enzymes (UDP glucuronosyltransferases, glutathione *S*-transferases, *N*-acetyltransferases, and sulfotransferases) have been shown to be functional in chimeric mice (Katoh et al., 2008). Because each chimeric mouse will reflect the liver profile and genetic makeup of the human donor’s hepatocytes, interindividual and ethnic differences in drug metabolism will undoubtedly exist. Nevertheless, variations of xenobiotic-metabolizing enzymes as well as other enzymes, receptors, transporters, transcription factors, and any other drug target located in human liver—might effectively be studied in such chimeric mouse lines.

Mammalian CYP1A1 basal mRNA is known to be negligible, resulting in no detectable CYP1A1 protein in any tissue, whereas basal levels of CYP1A2 mRNA and protein are relatively high in liver but generally low (protein undetectable on Western immuno-blot) in nonhepatic tissues; induction by a CYP1 inducer such as chemicals in cigarette smoke or

2,3,7,8-tetrachlorodibenzo-*p*-dioxin (**TCDD; dioxin**) increases CYP1A1 and CYP1A2 mRNA and protein levels (Eaton et al., 1995; Nebert et al., 2004). Recently, two humanized *CYP1A1_1A2* lines were compared with C57BL/6J (**B6**) inbred mice with regard to expression of CYP1A1 and CYP1A2 mRNA levels following TCDD pretreatment. Maximally-induced mRNA concentrations of mouse CYP1A1 were ~10 times higher in liver and lung and ~100-fold greater in kidney than those of human CYP1A1 (Shi et al., 2008). On the other hand, maximally-induced mRNA levels of mouse CYP1A2 in liver were <2-fold higher than those of human CYP1A2. Maximally-induced mRNA levels of human CYP1A2 in liver were ~12 times higher than those of human CYP1A1, whereas maximally-induced mRNA levels of mouse CYP1A2 in liver were ~3-fold greater than those of mouse CYP1A1 (Shi et al., 2008).

These data caused us to query how “physiologically” relevant these human mRNA levels might be, in the intact mouse. Do these humanized mouse lines actually reflect “average” *CYP1A1* and *CYP1A2* gene expression that might be expected among individuals in a human population, or is this expression abnormally low or high? One should be able to shed some light on this, by comparing precise copy numbers of basal and TCDD-induced CYP1A1 and CYP1A2 mRNA (combined with quantification of protein levels and enzyme assays) in the humanized mouse lines versus wild-type mice.

Those who oppose the use of laboratory animals, and recommend instead that everyone utilize cells in culture, often declare that studies with cultured cell lines can provide information that would accurately reflect what is found in the intact animal. We therefore have compared the above-mentioned CYP1A1 and CYP1A2 parameters in liver from the humanized mouse lines and wild-type mice with those in human versus mouse hepatoma-derived established cell culture lines. The present study addresses these questions. Answering these questions should be beneficial, before launching into human risk assessment studies using such humanized mouse lines.

Material and methods

Mice

C57BL/6J (B6) mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Development of the humanized *hCYP1A1_1A2_Cyp1a1/1a2(-/-)-Ahr^{b1}* transgenic line has been detailed (Dragin et al., 2007). Chimeric mice bearing human hepatocytes were generated using *uPA*(+/-)/*SCID* mice as the host (Giannini et al., 2003) and characterized (Tateno et al., 2004; Katoh et al., 2008); their human hepatocyte-replacement rates were between 73% and 83%. All experiments involving mice adhered to the Guidelines for Animal Experiments and Use Committee of the Nihon University School of Medicine.

Treatment of mice

Mice were treated with intraperitoneal TCDD (25 µg/kg for 24 h), versus corn oil vehicle alone for untreated. At least three groups (*N*=3 each time) were studied to ensure reproducibility.

Cell cultures and treatment

The human HepG2 established cell line was derived from a hepatoblastoma (Dearfield et al., 1983). The mouse Hepa-1c1c7 line was derived from a C57L/J hepatoma (Bernhard et al., 1973). Cultured cells were treated with 10 nM TCDD for 24 h before total RNA isolation.

Reverse transcription

Total RNAs from samples were prepared by the acid guanidine thiocyanate-phenol/chloroform method (Tavangar et al., 1990). The cDNAs were synthesized using the ImProm-II Reverse Transcription system (Promega, Madison, WI) (Inaba et al., 2007).

Quantitative real-time PCR (qRT-PCR)

We used the primers listed in Table 1. The qRT-PCR was performed in an ABI PRISM 7000 Sequence Detection System™ (Applied Biosystems), using Power SYBR Green PCR Master Mix (Applied Biosystems). Individual CYP1 mRNA abundance was determined, using the standard-curve method (from 10^1 to 10^8 copies/ μ L), as previously described by K. Livak (PE-ABI; Sequence Detector User; Bulletin #2) (Winer et al., 1999). Each sample was normalized to mouse glyceraldehyde-3-phosphate dehydrogenase (**GAPDH**) mRNA.

CYP1A mRNA copy numbers

Transcripts from the human *CYP1A1* and *CYP1A2* and the mouse *Cyp1a1* and *Cyp1a2* genes were quantified by fitting qRT-PCR data to a curve generated from cloned RNAs (cRNAs) for each CYP1. Briefly described, templates for cRNA synthesis were produced by PCR on cDNA constructs from each CYP1A cDNA that had been cloned into pcDNA3.1(+) (Invitrogen), using T7 RiboMAX™ Express Large-Scale RNA Production System (Promega) (Uno et al., 2006). The cRNAs were used to generate a standard curve in the PCR reactions from which mRNA copy numbers from qRT-RNA measurements could be extrapolated.

Western immunoblot analysis

Mice were euthanized by carbon dioxide asphyxiation followed by cervical dislocation. The liver was excised, and microsomes were prepared as previously described (Dalton et al., 2000). Protein concentrations were determined by the bicinchoninic acid method (Pierce Chemical Co.; Rockford, IL), according to details provided by the manufacturer. Microsomal proteins were separated on sodium dodecylsulfate (0.1%)–polyacrylamide (12%) minigels. Separated proteins were transferred to nitrocellulose membranes. Western immunoblot analysis was performed using goat polyclonal anti-rat CYP1A1/1A2 antibody; this antibody (Daiichi Pure Chemicals, Tokyo, Japan) recognizes both the human and mouse CYP1A1 and CYP1A2 proteins. We used alkaline phosphatase-conjugated secondary antibodies (Kirkegaard Perry Lab., Gaithersburg, MD) and the Alkaline Phosphatase Conjugate Substrate Kit™ (Bio-Rad Lab., Hercules, CA), with exposure times ranging from 5 to 10 min.

Enzyme assays

Determination of microsomal BaP hydroxylase (Nebert and Gelboin, 1968) and ethoxyresorufin *O*-deethylase (**EROD**) (Burke et al., 1977) activities principally represent CYP1A1 activity. Acetanilide 4-hydroxylase (Shertzer et al., 2001) and methoxyresorufin *O*-demethylase (**MROD**) (Berthou et al., 1992; Hamm et al., 1998; Shertzer et al., 2001) activities principally (but not exclusively) represent CYP1A2 activity. These enzymes were assayed by the methods cited. Although the MROD spectrophotofluorometric assay is sensitive and reliable, it has been demonstrated (Hamm et al., 1998) that the MROD assay is not the most suitable for estimating CYP1A2 activity. In *Cyp1a2*($-/-$) knockout mice, it was shown that hepatic MROD activity was increased 70-fold by TCDD treatment, indicating that other TCDD-inducible enzymes contribute to inducible MROD activity. In contrast, acetanilide 4-hydroxylase activity in *Cyp1a2*($-/-$) knockout mice was induced only 2-fold by dioxin (Shertzer et al., 2001), suggesting that it is by far the preferred enzyme activity for estimating CYP1A2 catalytic expression.

Biohazard precaution

TCDD is highly toxic and regarded as a likely human carcinogen. All personnel were instructed in safe handling procedures. Lab coats, gloves and masks were worn at all times, and contaminated materials were collected separately for disposal by the Hazardous Waste Unit or by independent contractors. TCDD-treated mice were housed separately, and their carcasses

regarded as contaminated biological materials. TCDD-treated cells in culture, and culture medium from these cells, were also regarded as contaminated biological materials.

Statistical analysis

Statistical significance between groups was determined by analysis-of-variance among groups and Student's *t*-test between groups. All assays were performed in duplicate or triplicate, and repeated at least twice. Statistical analyses were also carried out with the use of SAS[®] statistical software (SAS Institute Inc.; Cary, NC) and Sigma Plot (Systat Software, Inc., Point Richmond, CA).

Results and discussion

Factors affecting CYP expression

In a previous study of the entire gastrointestinal tract (Uno et al., 2008), large differences in basal but especially inducible CYP1A1 and CYP1A2 mRNA and protein levels were seen. This variability appears to depend on the route-of-administration and the target organ being studied: oral versus intraperitoneal administration of TCDD or BaP can drastically alter CYP1 mRNA levels in various cell types of the intestine, from tongue to colon (Uno et al., 2008). In two studies comparing humanized mice with wild-type controls (Dragin et al., 2007; Shi et al., 2008), large differences were also observed in human CYP1A1 or CYP1A2 mRNA, compared with mouse CYP1A1 or CYP1A2 mRNA. In the chimeric *uPA/SCID* humanized mouse, although CYP1A1 was not studied, large variability in CYP1A2 expression has also been seen (Katoh et al., 2008).

Reasons for differences in human transgene expression in humanized mouse tissues include: [a] genotype of the volunteer from whom the BAC library was derived (Jiang et al., 2005) or from whose hepatocytes were infused into a *uPA(+)/SCID* mouse (Katoh et al., 2008; [b] chromosomal location of the randomly inserted BAC transgene affecting transgene expression, *i.e.* the "neighborhood effect" (Bedell et al., 1996; Milot et al., 1996; Olson et al., 1996; Muller et al., 2001); [c] genetic background (modifier genes) of a particular inbred strain that can influence transgene expression (Bonyadi et al., 1997; Cranston and Fishel, 1999; Bennett et al., 2000); and [d] a BAC containing the human gene(s) (Jiang et al., 2005; Cheung et al., 2005) which does not include *trans*-regulatory, or all of the *cis*-regulatory, sites needed for "normal" expression of the transgene(s) in each mouse tissue or cell type studied.

Comparison of human versus mouse CYP1A1 mRNA levels in liver

Fig. 1A compares human and mouse CYP1A1 mRNA copy numbers in the *hCYP1A1_1A2_Cyp1a1/1a2(-/-)* line, B6 wild-type mice containing no human transgenes, chimeric *uPA/SCID* mice (**chimera**), and *uPA(+)/SCID* control mice containing no human hepatocytes (*uPA/SCID* mice). Human basal CYP1A1 mRNA levels in the liver of *hCYP1A1_1A2* and chimeric mice were quite low, having $\sim 1.3 \times 10^7$ and $\sim 1.2 \times 10^7$ transcript copy numbers (per μg total RNA), respectively; both were strikingly increased by TCDD to $\sim 7.3 \times 10^9$ and $\sim 2.0 \times 10^9$ copy numbers, respectively.

Mouse basal CYP1A1 mRNA levels in B6, chimeric, and *uPA/SCID* mice (Fig. 1A) were also quite low ($\sim 1.8 \times 10^6$, $\sim 1.7 \times 10^6$, and 1.0×10^6 copy numbers, respectively), but all were dramatically induced by TCDD to $\sim 2.5 \times 10^8$, $\sim 8.0 \times 10^7$, and $\sim 1.5 \times 10^8$ copy numbers, respectively. As expected, no human CYP1A1 mRNA was detected in B6 or *uPA/SCID* mice, and no mouse CYP1A1 mRNA was detected in the *hCYP1A1_1A2_Cyp1a1/1a2(-/-)* line.

Comparison of human versus mouse CYP1A2 mRNA levels in liver

Human basal CYP1A2 mRNA levels in *hCYP1A1_1A2_Cyp1a1/1a2*($-/-$) and chimeric mice (Fig. 1B) were low ($\sim 2.6 \times 10^8$ and $\sim 0.89 \times 10^8$ transcript copy numbers, respectively). Both were significantly elevated by TCDD to $\sim 9.7 \times 10^8$ and $\sim 7.7 \times 10^8$ copy numbers, respectively.

Mouse basal CYP1A2 mRNA concentrations in B6, chimeric, and *uPA/SCID* mice were also low ($\sim 2.2 \times 10^8$, $\sim 0.16 \times 10^8$ and $\sim 1.2 \times 10^8$ copy numbers, respectively); all three were significantly induced by TCDD to $\sim 4.2 \times 10^9$, $\sim 0.73 \times 10^9$, and 2.8×10^9 copy numbers, respectively (Fig. 1B). As expected, no human CYP1A2 mRNA was detected in B6 and *uPA/SCID* mice, and no mouse CYP1A2 mRNA was detected in the *hCYP1A1_1A2_Cyp1a1/1a2*($-/-$) line.

Comparison of human versus mouse CYP1A1 and CYP1A2 mRNA levels

It should be noted that the basal expression levels of human and mouse CYP1A2 mRNA ($1-3 \times 10^8$ copy numbers) were much higher (Fig. 1) than those of CYP1A1 mRNA ($\sim 10^7$ copy numbers). This conclusion supports the results of studies long ago (Nebert, 1989; Eaton et al., 1995). The induction of human and mouse CYP1A1 and CYP1A2 mRNAs by TCDD is also well known (Nebert, 1989; Eaton et al., 1995; Nebert et al., 2004).

A previous report (Shi et al., 2008) compared the expression of CYP1A1 and CYP1A2 mRNA in liver between two humanized *CYP1A1_1A2_Cyp1a1/1a2*($-/-$) lines and the B6 inbred mouse: maximally-induced mRNA levels of mouse CYP1A1 were described as ~ 10 times higher than those of human CYP1A1; in contrast, maximally-induced mRNA levels of mouse CYP1A2 were <2 -fold higher than those of human CYP1A2 in liver. However, the present study (in which we find a mouse/human induced CYP1A1 ratio of ~ 0.03 and a mouse/human induced CYP1A2 ratio of ~ 4) appears not to be consistent with this previous report.

The previous report also found that maximally-induced mRNA levels of human CYP1A2 in liver were ~ 12 times higher than those of human CYP1A1, whereas maximally-induced mRNA levels of mouse CYP1A2 were ~ 3 -fold greater than those of mouse CYP1A1 (Shi et al., 2008). In the present study, these ratios are 0.13 and 16.8, respectively. These large differences in the calculated ratios clearly reflect the disparity between the “relative values” given in the previous report and the “absolute values” (*i.e.* copy numbers per μg total RNA) in the present study.

Human induced and basal CYP1A2 mRNA copy numbers in chimeric mice were 73–80% lower than those in *uPA/SCID* mice (Fig. 1B). This decrease can easily be explained when the human hepatocyte-replacement rate (73%–83%) is taken into account. This finding supports the notion that human hepatocytes in chimeric mice liver are affected by TCDD independently from mouse hepatocytes, suggesting that human hepatocytes in chimeric mice liver can mimic those in human liver.

The induction rate (Fig. 1) of human CYP1A1 mRNA in the *hCYP1A1_1A2_Cyp1a1/1a2*($-/-$) line is quite remarkable (>500 -fold), whereas that in chimeric mouse was not nearly as high (~ 170 -fold). In contrast, the induction rate of human CYP1A2 mRNA in *hCYP1A1_1A2_Cyp1a1/1a2*($-/-$) mice was ~ 3.7 -fold, whereas that in the chimera was higher (~ 8.7 -fold). These differences in fold-induction could be due to differences in the transcription regulatory regions associated with each of the two genes—if we assume that human and mouse genomic regulatory motifs might differ in their ability to govern these two human transgenes. This might not be a valid assumption, however, because many transcription factors and their DNA-binding motifs are highly conserved among vertebrates and, indeed, in some cases down to the fly, worm and yeast.

The BAC carrying the human *CYP1A1_CYP1A2* locus includes the 23.3-kb bidirectional promoter, plus 56 kb 3'-ward of *CYP1A1* and 86 kb 3'-ward of *CYP1A2* (Jiang et al., 2005). The transgenes in the *hCYP1A1_1A2_Cyp1a1/1a2(-/-)* mouse thus would carry human *cis*-regulatory motifs only within these sequences responsible for TCDD up-regulation, whereas expression of the human *CYP1A1* and *CYP1A2* genes in chimeric mice should be controlled by any and all of the human *cis*- and *trans*-regulatory enhancers in the same way as they are in human liver hepatocytes.

The expression level of mouse CYP1A1 induced mRNA in B6 is comparable to that in *uPA/SCID* mice and might also be comparable to that in chimeric mice when the human hepatocyte-replacement rate is taken into consideration. A similar conclusion might also be reached if one compares the expression levels of mouse induced CYP1A2 mRNA among B6, chimeric, and *uPA/SCID* mice. As a whole, we conclude that the *hCYP1A1_1A2_Cyp1a1/1a2(-/-)* line and the human hepatocyte chimeric mouse show similar expression levels of basal mRNA for the human *CYP1A1* and *CYP1A2* genes. Likewise, there are similar expression levels of TCDD-induced mRNA for these two genes, although their extent of induction is variable.

Comparison of human versus mouse CYP1A1 and CYP1A2 protein levels in liver

Western immunoblots of liver were carried out from the four mouse types, control versus TCDD-pretreated (Fig. 2). The polyvalent antiserum was raised against rat CYP1A1/1A2 and thus is not likely to recognize equally the human and mouse CYP1A1 and CYP1A2 proteins; consequently, a strict quantitative comparison of the human versus mouse orthologous protein concentrations is not possible. This problem has been recognized before and discussed in detail (Jiang et al., 2005).

Comparison of human versus mouse CYP1A1 and CYP1A2 TCDD-induced enzyme activities in liver

For BaP hydroxylase and EROD as two activities associated pre-dominantly with CYP1A1, the correlations between enzyme activities (Fig. 3A) and mRNA levels (Fig. 1A) are extremely variable for BaP hydroxylase but quite consistent for EROD activity. Thus, B6 mice exhibit one-half as much TCDD-induced BaP hydroxylase activity (per unit of mCYP1A1 mRNA) as *uPA/SCID* mice (Table 2). The B6 mouse shows ~170 times more induced BaP hydroxylase activity (per unit of mCYP1A1 mRNA), compared with the *hCYP1A1_1A2_Cyp1a1/1a2(-/-)* mouse's induced BaP hydroxylase activity (per unit of hCYP1A1 mRNA). Chimeric mice exhibit ~6.2-fold more induced BaP hydroxylase activity (per unit of hCYP1A1 mRNA) than *hCYP1A1_1A2_Cyp1a1/1a2(-/-)* mice (Table 2). The *uPA/SCID* mouse shows ~42 times more induced BaP hydroxylase activity (per unit of mCYP1A1 mRNA), compared with the chimeric mouse's induced BaP hydroxylase activity (per unit of hCYP1A1 mRNA).

In contrast, B6 mice display about the same amount of TCDD-induced EROD activity (per unit of mCYP1A1 mRNA) as *uPA/SCID* mice (Table 2). The B6 mouse shows ~54 times more induced EROD activity (per unit of mCYP1A1 mRNA), compared with the *hCYP1A1_1A2_Cyp1a1/1a2(-/-)* mouse's induced EROD activity (per unit of hCYP1A1 mRNA). Chimeric mice exhibit ~1.7 times more induced EROD activity (per unit of hCYP1A1 mRNA) than *hCYP1A1_1A2_Cyp1a1/1a2(-/-)* mice (Table 2). The *uPA/SCID* mouse shows ~39 times more induced EROD activity (per unit of mCYP1A1 mRNA), compared with the chimeric mouse's induced EROD activity (per unit of hCYP1A1 mRNA).

Why does the humanized *hCYP1A1_1A2_Cyp1a1/1a2(-/-)* mouse carry so little enzyme activity toward BaP, compared with the chimeric mouse? This difference can be explained from the human hepatocyte-replacement rate (73%–83%) in chimeric mice. The liver of

chimeric mice carries 73%–83% human hepatocytes, which exhibit extremely low BaP hydroxylase activity.

For acetanilide 4-hydroxylase and MROD as two activities associated predominantly with CYP1A2, the correlations between enzyme activities (Fig. 3B) and mRNA levels (Fig. 1B) are very much consistent with one another. B6 mice show virtually the same amount of TCDD-induced acetanilide 4-hydroxylase activity (per unit of mCYP1A2 mRNA) as *uPA/SCID* mice (Table 2). The B6 mouse shows about the same amount of induced acetanilide 4-hydroxylase activity (per unit of mCYP1A2 mRNA), compared with the *hCYP1A1_1A2_Cyp1a1/1a2(-/-)* mouse's induced acetanilide 4-hydroxylase activity (per unit of hCYP1A2 mRNA). Chimeric mice exhibit twice as much induced acetanilide 4-hydroxylase activity (per unit of hCYP1A2 mRNA) than *hCYP1A1_1A2_Cyp1a1/1a2(-/-)* mice (Table 2). The chimeric mouse shows ~2.3-fold more induced acetanilide 4-hydroxylase activity (per unit of mCYP1A2 mRNA), compared with the *uPA/SCID* mouse's induced acetanilide 4-hydroxylase activity (per unit of hCYP1A2 mRNA).

B6 mice exhibit one-half as much TCDD-induced MROD activity (per unit of mCYP1A2 mRNA) as *uPA/SCID* mice (Table 2). The *hCYP1A1_1A2_Cyp1a1/1a2(-/-)* mouse shows ~2-fold more induced MROD activity (per unit of mCYP1A2 mRNA), compared with the B6 mouse's induced MROD activity (per unit of hCYP1A2 mRNA). The *hCYP1A1_1A2_Cyp1a1/1a2(-/-)* mice exhibit ~2.4 times more induced MROD activity (per unit of hCYP1A2 mRNA) than chimeric mice (Table 2). The *uPA/SCID* mouse shows twice as much induced MROD activity (per unit of mCYP1A2 mRNA), compared with the chimeric mouse's induced MROD activity (per unit of hCYP1A2 mRNA). Expression of CYP1A2 catalytic activity, relative to CYP1A2 mRNA levels, in the humanized *hCYP1A1_1A2_Cyp1a1/1a2(-/-)* and chimeric mouse lines is therefore very robust and within 2-fold similar to that expressed in mouse liver.

Comparison of human versus mouse CYP1A1 and CYP1A2 mRNA levels in hepatoma-derived cell culture lines

Animal rights' activists have urged scientists to study physiological functions in cell cultures rather than using live laboratory animals. Many studies have shown, however, that parameters found in cell culture do not accurately reflect what happens in the intact animal.

How does the expression of the *CYP1A1* and *CYP1A2* genes in intact liver compare with that in hepatoma-derived established cell lines? In HepG2 cells (Fig. 4A), human basal CYP1A1 mRNA was negligible, whereas human TCDD-induced CYP1A1 mRNA gave $\sim 5.4 \times 10^9$ copy numbers (per μg total RNA). In Hepa-1c1c7 cells (Fig. 4A), mouse basal versus TCDD-induced CYP1A1 mRNA showed $\sim 0.35 \times 10^8$ and $\sim 1.9 \times 10^8$ copy numbers, respectively. Mouse CYP1A1 mRNA was not detected in HepG2, and human CYP1A1 mRNA was not detected in Hepa-1c1c7 cells.

In HepG2 cells (Fig. 4B), human basal versus TCDD-induced CYP1A2 mRNA gave $\sim 0.27 \times 10^6$ and $\sim 4.8 \times 10^6$ copy numbers, respectively. In Hepa-1c1c7 cells (Fig. 4B), mouse basal versus TCDD-induced CYP1A2 mRNA showed $\sim 0.14 \times 10^6$ and $\sim 1.2 \times 10^6$ copies, respectively. Mouse CYP1A2 mRNA was not detected in HepG2, and human CYP1A2 mRNA was not detected in Hepa-1c1c7 cells.

Thus, in livers of the *hCYP1A1_1A2_Cyp1a1/1a2(-/-)* and chimeric mice, the copy number of human induced CYP1A1 mRNA is 7.5 and 2.6 times, respectively, greater than that of human induced CYP1A2 mRNA. On the other hand, in the HepG2 liver-derived established cell line, the copy number of human induced CYP1A1 mRNA is more than 1100 times greater than that of human induced CYP1A2 mRNA. In livers of the B6 and *uPA/SCID* mice, the copy

number of mouse induced CYP1A2 mRNA is 40-fold and 20-fold, respectively, greater than that of mouse induced CYP1A1 mRNA; in contrast, in the Hepa-1c1c7 established cell line, the copy number of mouse induced CYP1A1 mRNA is almost 1600-fold greater than that of mouse maximally-inducible CYP1A2 mRNA. This decline in *CYP1A2* gene expression seen in established cell lines reflects the well-known fact that numerous “housekeeping” genes such as *CYP1A2* are extinguished, or are greatly decreased in expression—in tumor cells as well as “established”, or transformed, cell lines in culture (Owens et al., 1975; Nebert, 2006). However, such suppression often does not occur for the *CYP1A1* gene in differentiated tumors, including the HepG2 and Hepa-1c1c7 hepatoma-derived cell lines (Owens et al., 1975; Nebert, 2006).

Comparison of human versus mouse CYP1A1 and CYP1A2 protein levels in hepatoma-derived cell culture lines

We carried out Western immunoblots of Hepa-1c1c7 and HepG2 cells, control versus TCDD-pretreated (Fig. 5). The human CYP1A1 protein appears to migrate more rapidly than the mouse CYP1A1 protein. We believe the level of CYP1A2 protein was so low that it was not detected in either established hepatoma cell line.

Comparison of human versus mouse CYP1A1 and CYP1A2 TCDD-induced enzyme activities in hepatoma-derived cell culture lines

Different from what was found in mouse liver, the correlations between enzyme activities (Fig. 6A) and mRNA levels (Fig. 4A) are extremely variable for EROD activity but more consistent for BaP hydroxylase activity. Hepa-1c1c7 cells show ~4.8 times more TCDD-induced BaP hydroxylase activity (per unit of mCYP1A1 mRNA) than HepG2 cells exhibit for induced BaP hydroxylase activity (per unit of hCYP1A1 mRNA) (Table 3). Hepa-1c1c7 cells show ~1500 times more TCDD-induced EROD activity (per unit of mCYP1A1 mRNA) than HepG2 cells exhibit for induced EROD activity (per unit of hCYP1A1 mRNA). For whatever reason, HepG2 cells do not display very high induced BaP hydroxylase activity, and their induced EROD activity is extremely low.

For acetanilide 4-hydroxylase and MROD as two activities associated predominantly with CYP1A2, the correlations between enzyme activities (Fig. 6B) and mRNA levels (Fig. 6B) are better than those with CYP1A1. Hepa-1c1c7 cells show ~16-fold more TCDD-induced acetanilide 4-hydroxylase activity (per unit of mCYP1A2 mRNA) than HepG2 cells exhibit for induced acetanilide 4-hydroxylase activity (per unit of hCYP1A2 mRNA) (Table 3). Hepa-1c1c7 cells show ~15-fold more TCDD-induced MROD activity (per unit of mCYP1A2 mRNA) than HepG2 cells exhibit for induced MROD activity (per unit of hCYP1A2 mRNA). Therefore, HepG2 cells do not express either CYP1A1 or CYP1A2 activities nearly as robustly as do Hepa-1c1c7 cells.

Conclusions

In this study we have measured the amount of variability between human and mouse CYP1A mRNA and protein levels and corresponding enzyme activities in the humanized *hCYP1A1_1A2_Cyp1a1/1a2(-/-)* and chimeric *uPA/SCID* lines, by comparing these parameters with those seen in wild-type mice from which these two lines were derived. We have also compared these mRNA and protein levels and corresponding enzyme activities in mouse hepatoma-derived Hepa-1c1c7 and human hepatoblastoma-derived HepG2 established cell culture lines. Clearly, the CYP1A1/CYP1A2 activity ratios in these hepatoma-derived established cell lines are not accurate indicators of those in liver from the intact mouse. Undoubtedly, this discrepancy is primarily caused by the dramatically lowered CYP1A2 mRNA levels—presumably due to “extinction” of the normal expression of the *CYP1A2* gene in these hepatoma-derived established cell lines. Not only very low CYP1A2 enzyme activity

per unit of mRNA was seen in both Hepa- 1c1c7 and HepG2 cells, but also low CYP1A1 enzyme activity per unit of hCYP1A1 mRNA was found in HepG2 cells.

Comparing liver of the two humanized mouse lines with liver of mice from which these two humanized lines were derived was most disturbing when one examined CYP1A1-specific (BaP and ethoxyresorufin) and CYP1A2-specific (acetanilide and methoxyresorufin) substrates metabolized—per unit of mCYP1A1, hCYP1A1, mCYP1A2 or hCYP1A2 mRNA. The hCYP1A1 in mouse liver was between 38 and more than 170 times less efficient than mCYP1A1 in the hydroxylation of BaP and about 54-fold less efficient in EROD activity. In contrast, hCYP1A2 in mouse liver appeared to function nearly equivalent to mCYP1A2 in wild-type mouse liver.

The levels of human CYP1A1 and CYP1A2 mRNA in both humanized mouse lines appear to be quite compatible with what might be expected among individual persons in any human population. It is very clear, however, that substrate specificity varies widely, independent of human versus mouse CYP1A1/1A2 mRNA or protein concentrations. Nevertheless, keeping this caveats in mind, both of these lines should still be useful for studies in human risk assessment, toxicology, pharmacology, and other medical subspecialties.

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Fig. 1.

Human (*upper panels*) versus mouse (*lower panels*) CYP1A1 (A) and CYP1A2 (B) mRNA copy numbers in liver from the *hCYP1A1_1A2_Cyp1a1/1a2(-/-)_Ahr^{bl}* mouse line, B6 inbred mouse, chimeric mouse, and *uPA/SCID* mouse—with, versus without, TCDD pretreatment. When administered, TCDD (25 μg/kg body weight 24 h before killing) was given intraperitoneally. “ND” (nondetectable by qRT-PCR) denotes nothing above background, whereas absence of “ND” (detectable, but extremely low by qRT-PCR) denotes something measurable above background. On Y-axis: **hCYP1A1** or **hCYP1A2**=human mRNA; **mCYP1A1** or **mCYP1A2**=mouse mRNA. For this figure and Fig. 4, the method for determining the copy number of mRNA molecules per μg total RNA is given in “Materials & methods”. Note the different labels on the Y-axes of these figures. *Bars and brackets* denote means±S.E.M., respectively (*N*=3 independent experiments).

Fig. 2.

Western immunoblot analysis of mouse versus human hepatic CYP1A1 and CYP1A2 proteins in the same mouse lines as in Fig. 1, using a polyclonal antibody that recognizes both mammalian CYP1A1 and CYP1A2. TCDD-induced mouse and human CYP1A1 proteins are both ~56.0 kDa, whereas TCDD-induced mouse and human CYP1A2 proteins are both ~54.5 kDa. Lanes 1–2 represent human CYP1A proteins only, whereas lanes 5–6 represent ~78% human CYP1A proteins and ~22% mouse CYP1A proteins. Lanes 3–4 and 7–8 depict only mouse CYP1A proteins. We used β-actin mRNA as a control for standardizing the amount of protein loaded per lane. The amount of microsomal protein (10 μg) loaded per lane was constant for all lanes.

Fig. 3. (A) BaP hydroxylase and EROD activity (both representing largely CYP1A1), and (B) acetanilide 4-hydroxylase and MROD activity (both representing largely CYP1A2) in liver microsomes from the same mouse lines as in Fig. 1. FU, fluorescent units. * $P<0.05$ and ** $P<0.01$, when comparing TCDD-pretreated with no pretreatment.

Fig. 4. Human (*upper panels*) versus mouse (*lower panels*) CYP1A1 (A) and CYP1A2 (B) mRNA copy numbers in mouse Hepa-1c1c7 cells and human HepG2 cells—with, versus without, TCDD exposure (10 nM for 24 h) in culture. Abbreviations are the same as those in Fig. 1.

Fig. 5.

Western immunoblot analysis of mouse versus human hepatic CYP1A1 and CYP1A2 proteins in the same cell culture lines as in Fig. 4. Everything is the same as that described for the Western blot in Fig. 2. The amount of cell culture protein (10 μ g) loaded per lane was constant for all lanes.

Fig. 6. (A) BaP hydroxylase and EROD activity (both representing largely CYP1A1), and (B) acetanilide 4-hydroxylase and MROD activity (both representing largely CYP1A2) in the same cell culture lines as in Fig. 4. * $P < 0.05$ and ** $P < 0.01$, when comparing TCDD-pretreated with no pretreatment.

Table 1

Primer pairs used in qRT-PCR.

Gene	Forward primer	Reverse primer
<i>hCYP1A1</i>	5'-AAGGGGCGTTGTGTCTTTGT-3'	5'-ATACACTTCCGCTTGCCCAT-3'
<i>hCYP1A2</i>	5'-ACAAGGGACACAACGCTGAA-3'	5'-AGGGCTTGTTAATGGCAGTG-3'
<i>mCyp1a1</i>	5'-CCTCATGTACCTGGTAACCA-3'	5'-AAGGATGAATGCCGGAAGGT-3'
<i>mCyp1a2</i>	5'-AAGACAATGGCGGTCTCATC-3'	5'-GACGGTCAGAAAGCCGTGGT-3'
<i>mGapdh</i>	5'-TGCACCACCAACTGCTTAG-3'	5'-GATGCAGGGATGATGTTC-3'

h, human; m, mouse.

Table 2

Ratios of mouse liver TCDD-induced enzymic activities per unit of mRNA*.

	mCYP1A1		hCYP1A1	
B6 mouse	7600 ± 2700	h1A1_1A2	44 ± 13	BaP hydroxylase
uPA/SCID	11,400 ± 3000	Chimera	270 ± 62	
B6 mouse	230 ± 64	h1A1_1A2	4.3 ± 1.3	EROD activity
uPA/SCID	290 ± 95	Chimera	7.4 ± 3.1	
	mCYP1A2		hCYP1A2	
B6 mouse	490 ± 84	h1A1_1A2	600 ± 220	Acetanilide
uPA/SCID	510 ± 200	Chimera	1200 ± 390	4-hydroxylase
B6 mouse	5.4 ± 1.0	h1A1_1A2	14 ± 5.5	MROD activity
uPA/SCID	13 ± 5.2	Chimera	5.6 ± 0.3	

* For BaP hydroxylase, these ratios represent FU/min/mg protein divided by mRNA×10⁹ per µg total RNA. For the other three enzyme activities, these ratios represent pmol/min/mg protein divided by mRNA×10⁹ per µg total RNA. Values are expressed as means ± S.E.

Table 3

Ratios of hepatoma-derived cell line TCDD-induced enzymic activities per unit of mRNA *.

mCYP1A1		hCYP1A1		
Hepa-1c1c7	55 ± 21	HepG2	11 ± 2.5	BaP hydroxylase
Hepa-1c1c7	210 ± 48	HepG2	0.14 ± 0.06	EROD activity
mCYP1A2		hCYP1A2		
Hepa-1c1c7	27,000 ± 2400	HepG2	1800 ± 930	Acetanilide 4-hydroxylase
Hepa-1c1c7	3700 ± 480	HepG2	250 ± 85	MROD activity

* For BaP hydroxylase, these ratios represent FU/min/mg protein divided by mRNA×10⁹ per µg total RNA. For the other three enzyme activities, these ratios represent pmol/min/mg protein divided by mRNA×10⁹ per µg total RNA. Values are expressed as means±S.E.