Review Article

Factors Influencing Regulation of CYP2B Expression

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ABSTRACT

Cytochrome P450 (P450) enzymes participate in a wide array of metabolic reactions. Of these P450s, CYP2B subfamily plays an important role in the metabolism of endogenous compounds and xenobiotics. CYP2B1 and CYP2B2 in rats and CYP2B9 and CYP2B10 in mice are major CYP2B isoenzymes constitutively and inducible expressed. Their constitutive expression is sexually dimorphic, specifically more expression in male than female rats, and more in female than male mice. Recent studies have shown that regulation of CYP2B expression is markedly influenced by not only various endogenous and exogenous compounds, but also age, sex, strain, and nutritional status. Regarding regulation of P450 expression in mouse liver, a C57BL/6 strain is one of the most suitable mouse models because of its marked response to CYP2B induction. The regulation of sexual dimorphism of CYP2B highly depends on numerous endogenous hormones including glucocorticoids, sex hormones and growth hormones. Adrenalectomy suggested that glucocorticoids induced CYP2B10 but simultaneously suppressed CYP2B9 expression in both sexes. β-estradiol (ES) up-regulated the expression of CYP2B9, while testosterone showed reverse activity of ES. Hypophysectomy and the age-expression profile revealed that growth hormone (GH) exerts suppressive effect on regulation of CYP2B9 and CYP2B10 expression in the males, but only on CYP2B10 in the females. Xenobiotics, i.e., phenobarbital, dexamethasone, DDT (1, 1, 1-trichloro-2, 2-bis (*p*-chlorophenyl) ethane), are exogenous factors influencing the CYP2B expression. For example, phenobarbital and DDT induce both CYP2B9 and CYP2B10 while dexamethasone predominantly induces CYP2B10, but simultaneously suppresses CYP2B9. Therefore, the factors that affect regulation of CYP2B expression should be thoroughly considered to eliminate their confounding effects, leading to accurate and precise outcome measures.

Keywords: CYP2B, C57BL/6, sexual dimorphism, endogenous hormones, xenobiotics

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Introduction

It is unavoidable for all organisms to expose foreign chemicals, or xenobiotics. These xenobiotics, both manufactured and natural in nature, include drugs, industrial chemicals, pesticides, pollutants, secondary plant metabolites, and toxins produced by molds, plants, and animals. The physical property that enables many xenobiotics to be absorbed through the skin, lung, or gastrointestinal track, namely lypophillicity, is an obstacle to their elimination, because lipophillic compound is readily reabsorbed.[1] Consequently, elimination of xenobiotic often depends on their conversion to water-soluble chemicals by process known as biotransformation or metabolism. Drug is one of important xenobiotics. In general, drug metabolizing reactions are divided into phase I and phase II reactions. Phase I reaction involves chemical alteration of drug structure by oxidation, reduction, or hydrolysis. In phase II reaction, the drug molecule is conjugated by glucuronidation, sulphation, or acetylation.[2] Cytochrome P50 superfamily (P450) is an important enzyme system in phase I of drug metabolism. P450s require a cytochrome P450 reductase to transfer electron from NADPH to their substrate (Figure 1). This process converts dugs or chemical compounds to water-soluble compounds to be excreted in urine or feces.[3] P450s, which are heme-containing protein, are found in the endoplasmic reticulum and mitochondria. In human, they are

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synthesized predominantly in the liver and, to a lesser extent, in the small intestine, kidney, adrenal, and other sites. While the P450 enzymes have extremely broad substrate and product specificities, an individual enzyme often exhibits a high specificity toward a certain substrate.[4] CYP2B is a large subfamily that encodes versatile catalysts of xenobiotics and steroid hydroxylation. Even closely related isoforms display distinct sex- and tissue-specific regulation.[5] A hallmark for *CYP2B* gene regulation is the strong inducibility of some isoforms by structurally diverse xenobiotics, including industrial solvent, barbiturates, antimycotics, and pesticides.[6] These chemicals are typified by phenobarbital (PB) and they can up-regulate several hepatic enzymes involved in xenobiotic metabolism and other genes as well.[7]

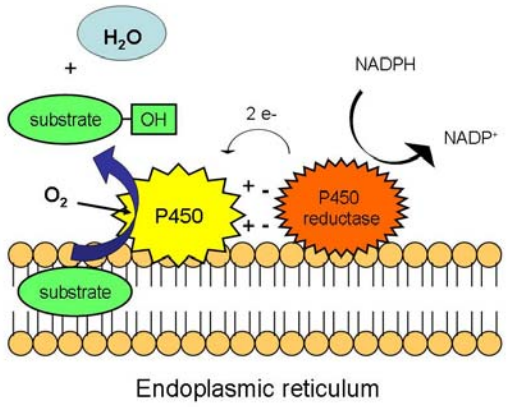

Figure 1 The cytochrome P450 system.

Rolf of CYP2B in Xenobiotic Metabolism

CYP2B6 which belongs to CYP2B subfamily has been studied extensively in many species. CYP2B6 appears to be the functional gene expression in human.[8] This enzyme catalyzes many reactions such as the *O*-dealkylation of 7-ethoxy-4-trifluoromethylcoumarin, the *N*-demethylation of benzphetamine, and the *O*-dealkylation of benzyloxyresorufin. However these reactions are not selective for CYP2B6. CYP2B6 plays a role in the metabolism of endogenous substrates including testosterone steroid.[9] Phenobarbital (PB) primarily induces the corresponding CYP2B enzymes in other species. For example CYP2B9/10, CYP2B1/2, CYP2B11, and CYP2B17 are the major phenobarbital-inducible P450 isoforms in mouse, rat, dog, and cynomolgus monkey, respectively. *CYP2B* genes are regulated physiologically by thyroids hormones, glucocorticoids, and growth hormone[8], which all remain under the control of the central nervous system.[10] At molecular level, the nuclear receptors-constitutive androstrane receptor (CAR), pregnane X receptor (PXR), and glucocorticoid receptor (GR) participate in their regulation.[11]

Regulation of CYP2B Expression

Besides nuclear receptors, level of drug metabolizing enzymes are influenced by a numerous factors, such as age, genetic background, physiologic state, disease, nutritional status as well as exposure to drugs, hormones, and environmental chemicals. Hence, factors that affect sexual dimorphic expression of CYP2B including sex, age, strain as well as some endogenous hormones and xenobiotics are discussed as follows.

The Difference of Strains

A vertebrate model commonly used as animal model is mouse because of their availability, size, low cost, ease of handling, and high reproduction rate.[12] Because of these reasons, mouse models were used for genetic analysis of CYP2B9 expression. PB has served as a prototype for a large group of structurally and functionally diverse xenochemicals that induce *CYP2B* gene.

Damon et al investigated the effect of genetic background on PB-inducibility of CYP2B9 and CYP2B10 mRNA expression by semi-quantitative specific RT-PCR in five inbred mouse strains including A/J, BALB/cByJ, C57BL/6J, DBA/2J, and SWR/J.[13] Male mice constitutively expressed CYP2B9 and CYP2B10 mRNAs, but a number of differences in their response to PB were observed. In all these mouse strains, PB induced CYP2B10 mRNA whereas it had either a positive or a negative effect on CYP2B9 expression, depending on strain and sex of the mice.

The results from Figure 2 suggested that the genetic basis for PB-induction in mice depended on the target gene, and that more than one regulatory step would be involved in this response pathway.

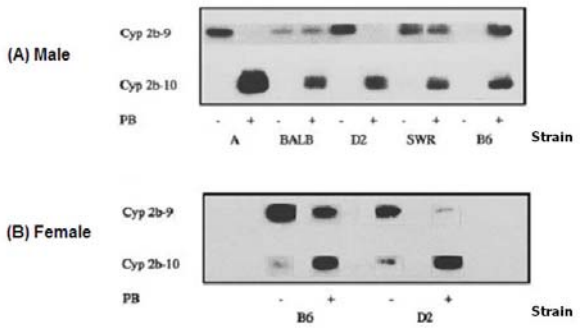

Figure 2 Regulation of CYP2B9 and CY2B10 mRNAs by Phenobarbital (PB) in male and female mice.[13] (A) RT-PCR analyses on 250 ng (CY2B9) and 100 ng (CYP2B10) of total RNA for both control and PB-treated animals. PCR products were analyzed by Southern blotting and hybridized with a full-length rat CYP2B1 cDNA probe. (B) CY2B9 PCR products were obtained from 50 ng (untreated and PB-treated D2 mice), or 100 ng (untreated and PB-treated B6 mice) of total RNA. CYP2B10 PCR products were obtained from 100 ng of total RNA. PCR products were also analyzed by Southern blotting and hybridized with a full-length rat CYP2B1 cDNA probe.

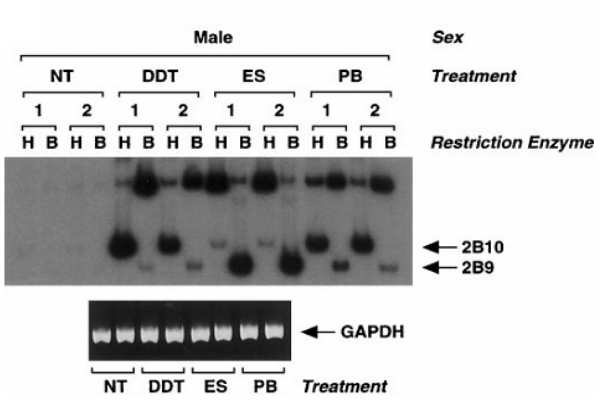

Figure 3 Induction of hepatic CYP2B9 and CYP2B10 mRNA expression by P450 inducers in C57BL/6 mice.[14] Adult C57BL/6 mice were daily subcutaneously treated with DDT at 100 mg/kg/day for 3 days, or ES at 0.5 mg/kg/day for 7 days, as well as PB intraperitoneally at 100 mg/kg/day for 3 days. The animals were killed 24 h after the last injection to prepare total RNA. Discrimination analysis of expressed CYP2B9 and CYP2B10 mRNA by RT-PCR. **Note:** NT, no treatment; DDT, 1,1,1-trichloro-2,2-bis(*p*-chloro phenyl) ethane; ES, β-estradiol benzoate; PB, phenobarbital sodium; H, *Hha*I; B, *Bgl*II; PCR 28 cycles; 1 and 2 indicate individual animals.

The regulatory mechanism behind the expression of CYP2B9 mRNA, a female-specific species inducibly expressed in the mouse liver, has yet to be elucidated. A potent inducer is needed to investigate the regulatory pathway of CYP2B9 expression. The next study of Jarukamjorn et al investigated the inducible expression of CYP2B subfamily in male C57BL/6 (B6) and DBA/2 (D2) mice, as well as their hybrids (B6D2F1) at the mRNA level by using semi-quantitative RT-PCR.[14] The result demonstrated that the expression of hepatic CYP2B mRNAs in the B6 was lightly induced by β-estradiol (ES), while that by PB or 1,1,1-trichloro2,2-bis (*p*-chlorophenyl) ethane (DDT) was prominent. Discriminating analysis showed a novelty that ES markedly induced CYP2B9 mRNA expression, whereas PB and DDT increased CYP2B10 more than CYP2B9 expression: albeit both mRNA species responded to all three inducers (Figure 3). Especially, the specific induction by ES of CYP2B9 mRNA in B6 male mice, but not D2 male mice, suggested strain dependency in the regulatory pathway of CYP2B9 expression.

The Influence of Endogenous Hormones

Of the endogenous factors studied, hormones appear to have considerable influence on the expression or activity of CYP2B enzymes. *CYP2B* genes are regulated physiologically by thyroid hormones, glucocorticoids, and growth hormone. Thus endogenous hormones are candidates in activating or depressing *CYP2B* gene expression.[15]

The first example study by Nemoto and Sakurai investigated the effects of growth hormone (GH) and glucocorticoid on the expression of CYP2B9/10 mRNA in C57BL/6NCrj mice-hepatocytes by RT-PCR.[15] They found that treatment with PB increased the expression of CYP2B10 more than CYP2B9 in both sexes. β-estradiol also induced both gene expressions in male liver, but more effect on CYP2B10 than CYP2B9 was observed. In the female liver, β-estradiol and testosterone slightly increased the levels of CYP2B10, whereas the expression of CYP2B9 was reduced (Figure 4). Dexamethasone induced more expression of more CYP2B10 mRNA than that of CYP2B9. Ovarectomy did not reduce the expression of these two mRNAs, but

rather increased them and the PB-inducible and non-inducible expressions of CYP2B9/10 mRNA were very slightly inhibited by GH regardless of sex.

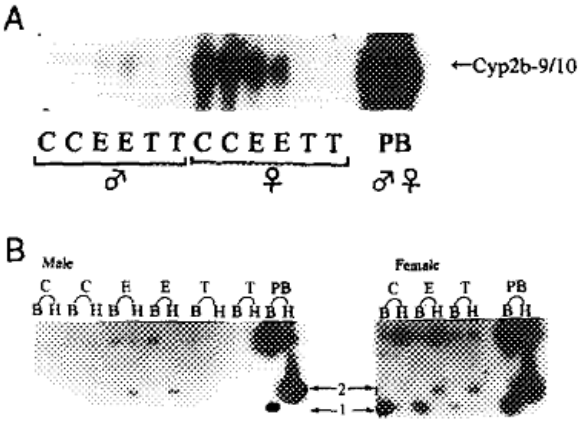

Figure 4 Expression of CYP2B9/10 mRNA in mouse liver.[15] Male and female C57BL/6 mice were injected with 400 μg testosterone propionate or 10 μg β-estradiol or 100 mg/kg phenobarbital. The animals were killed 24 h after the last injection. (A) Total liver RNA was Northern-blotted using a cDNA probe for CYP2B10. (B) One microgram of total RNA was reverse-transcribed and then amplified. **Note:** C, control ; E, β-estradiol; T, testosterone; PB, phenobarbital; B, *Bgl*II; H, *Hha*I;1, CYP2B9; 2, CYP2B10.

Jarukamjorn et al demonstrated the constitutive and inducible expression of CYP2B9/10 in mouse liver and culture hepatocytes.[6] The results showed that PB induced both CYP2B9 and CYP2B10 while dexsamethasone predominantly induced CYP2B10, but simultaneously suppressed CYP2B9 (Figure 5). Adrenalectomy increased the expression of CYP2B9 and CYP2B10 mRNAs, especially that of CYP2B9 in the male liver. In addition, the expression of one unknown species, which was constitutively suppressed, was increased in adrenalectomized male mice (Figure 6). The treatment of dexamethasone or adrenalectomy altered the expression of CYP2B subfamilies suggesting that endogenous glucocorticoid hormone plays a basic role in the constitutive expression of cytochrome P450.

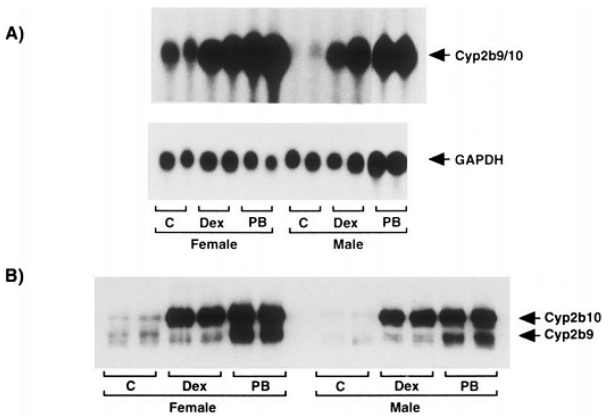

Figure 5 Expression of CYP2B9 and CYP2B10 in the mouse liver.[6] Male and female C57BL/6 mice were injected three times with 10 mg dexamethasone or 100 mg phenobarbital/ kg/day. The animals were killed 24 h after the last injection. (A) Total RNAs were Northern-blotted using a cDNA probe for Cyp2b10. (B) Western blotting of the hepatic microsomal proteins. **Note:** C, control; Dex, dexamethasone; PB, phenobarbital.

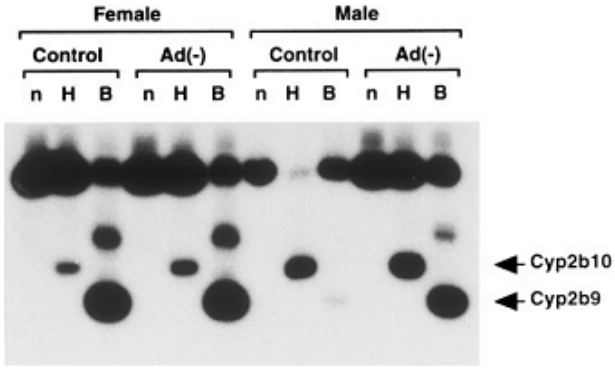

Figure 6 Expression of CYP2B9 and CYP2B10 mRNAs after adrenalectomy.[6] Four-week-old C57BL/6 mice were adrenalectomized and killed 5 days later. Total RNA was prepared from the liver and amplified by RT-PCR. **Note:** Ad (-), adrenalectomized mice. PCR cycles: male, 28; female, 20; n, none; B, BglII; H, HhaI.

Furthermore, the sex-related difference in the expression of CYP2B9 and CYP2B10 suggested that sex-dependent secretion of endogenous modulating factors was involved in the regulatory pathway.

Jarukamjorn et al examined sexual dimorphism in mouse CYP2B by investigating the developmental modification of the expression during maturation stage and by determining

the role of growth and sex hormones on the regulatory mechanism of this enzymes, using northern hybridization and RT-PCR.[16] The results revealed that during the prepubertal stage, the constitutive expression of hepatic CYP2B mRNA was similar in both sexes (Figure 7A). The expression was increased in pubertal female mice, whereas those in males gradually declined to nearly undetectable levels after puberty and CYP2B9 mRNA was the principal isoform expressed during prepuberty in both sexes (Figure 7B). During the maturation stage, CYP2B10 was expressed in both sexes, while CYP2B9 was diminished markedly in the male, resulting in a sexually dimorphic expression in adult mice. Hypophysectomy eliminated the sexual dimorphism in the mouse CYP2B subfamily by markedly increasing the expression of both CYP2B9 and CYP2B10 in males to levels similar to those in females (Figure 8). On the other hand, expression of CYP2B9 in females was almost unchanged, even after hypophysectomy. Thus the ratio of CYP2B9/CYP2B10 mRNA was increased significantly in male but not female mice.

Treatment of hypophysectomized mice of both sexes with sex hormones reduced the ratio of CYP2B9/CYP2B10 according to the increase of CYP2B10, except in the case of testosterone-treated hypophysectomized male mice (Table 1). This result indicates the possibility that estrogen can regulate *CYP2B10* gene expression. The results suggest that beside glucocorticoid hormones, the pituitary factor may be one of the expressions of the CYP2B subfamily in mouse liver.

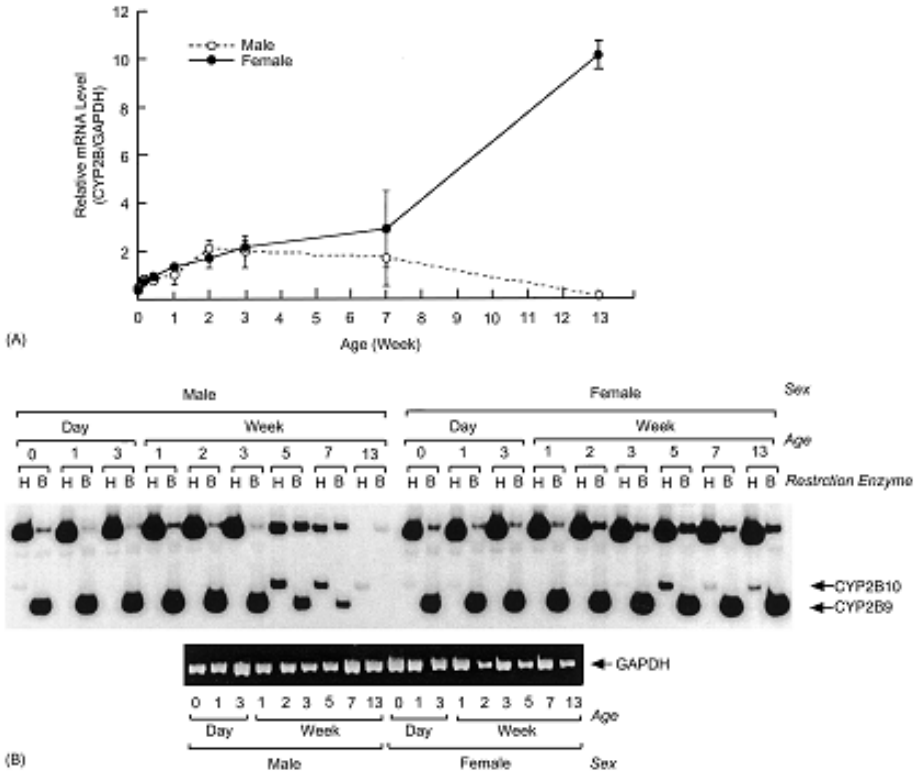

Figure 7 Developmental profile of CYP2B mRNA expression during maturation.[17] ddY mice of both sexes were killed at the indicated ages. (A) 10 micrograms of total hepatic RNA was northern-blotted and hybridized using a cDNA probe for *CYP2B10* gene. The blots were normalized to the signal for GAPDH mRNA. The relative mRNA level was determined densitometrically from autoradiographs and presented with age as a line graph (N = 3: mean ± SD). (B) Discrimination of the developmental expression of CYP2B9 and CYP2B10 mRNA by RT-PCR. Restriction enzymes: B, *Bgl*II; H, *Hha*I. A representative experiment of three is shown.

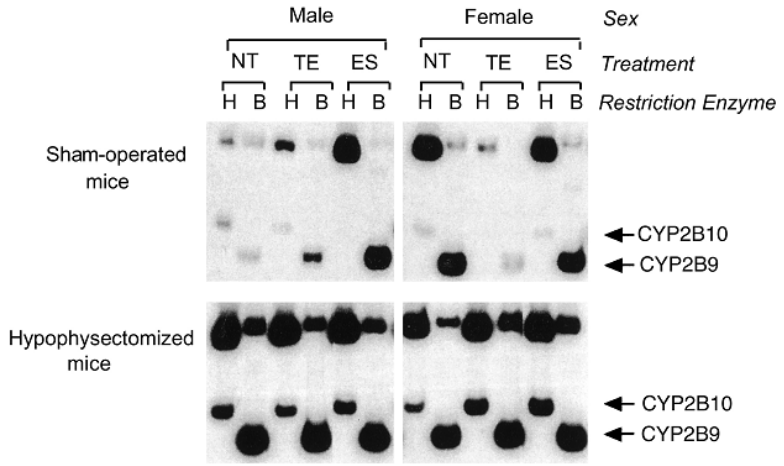

Figure 8 Modification of CYP2B mRNA expression by hypophysectomy and sex hormone supplementation.[16] 17β-Estradiol benzoate (ES) or testosterone propionate (TE) was administrated subcutaneously to hypophysectomoized or sham-operated C57BL/6 mice of both sexes. The mice were killed 24 h after the last injection. **Note:** NT, no treatment; Restriction enzymes: B, *Bgl*II; H, *Hha*I. A representative experiment of three is shown.

Table 1 Modification of CYP2B mRNA expression by hypophysectomy and sex hormone supplementation[16)].

Operation	Treatment	Relative expression					
		Male			Female		
		CYP2B9	CYP2B10	Ratio	CYP2B9	CYP2B10	Ratio
Sham-operation	No treatment	1.05 ± 1.28	0.96 ± 0.66	1.09	11.89 ± 0.58	1.00 ± 0.05	11.89
Hypophysectomy	No treatment	8.03 ± 0.48*	1.65 ± 0.11	4.87	10.94 ± 2.08	1.61 ± 0.51	6.79
	Estradiol	7.64 ± 0.57	2.31 ± 0.75	3.31	10.54 ± 1.22	3.67 ± 0.75**	2.87
	Testosterone	7.83 ± 0.51	1.53 ± 0.48	5.12	11.03	3.50***	3.15

Results are expressed as mean ± SD or as the mean of radioactivity from 3 or 2 individual mice per group, respectively.

* Significantly different from the non-treated sham-operated group ($P < 0.01$).

** Significantly different from the non-treated hypophysectomized group ($P < 0.01$).

*** Significantly different from the non-treated hypophysectomized group ($P < 0.05$).

Conclusions and Perspectives

The *P450* genes encode a superfamily of heme-thiolate proteins responsible for the oxidative metabolism of chemically diverse compounds of both endogenous and exogenous origin. The toxicity of certain xenobiotics, such as mutagents and carcinogens, is enhanced by P450-dependent metabolism in some cases.[17] CYP2B subfamily accounts for one of the P450s expressed in the liver which participates in the metabolism of endogenous hormones and xenobiotics[18] and one of the prototypic examples of sex-dependent differences in hepatic profiles.[16] There are many factors that can affect the regulation of the CYP2B expression such as age, gender, genetic background, physiologic state, disease, and nutritional status as well as exposure to drugs, hormones, and environmental chemicals[1]. A mouse model commonly used as animal model because of their availability, size, low cost, ease of handing, and high reproduction rate.[12] The study on expression of CYP2B among 5 mouse strains including A/J, BALB/c, C57BL/6J, DBA/2J, and SWR/J by phenobarbital showed that CYP2B10 was inducible in all 5 mouse strains while CYP2B9 was inducible in most mouse strains except DBA/2.[13] Consideration on regulation of CYP2B expression in mouse liver, a C57BL/6 strain is one of the most suitable mouse models because of its high response to CYP2B inducers. The regulation of sexual dimorphism of CYP2B highly depends on numerous endogenous hormones including

glucocorticoids, sex hormones and growth hormones. Glucocorticoid hormones which are produced by adrenal gland, suppressed the CYP2B9 expression in both sexes. Simultaneous induction of CYP2B10 with suppression of CYP2B9 mRNAs by the treatment of dexamethasone, a synthetic glucocorticoid hormone, as well as an increase of CYP2B9 expression level in adrenalectomized mice supported that glucocorticoid hormones function as a key suppressor on CYP2B9 mRNA. A female sex hormone (estradiol) increased the level of CYP2B9 expression, whereas the level of this enzyme was reduced by a male sex hormone (testosterone). Hypophysectomy eliminated the sexual dimorphism in CYP2B subfamily by increasing the expression of both CYP2B in the males to nearly the same level as that in the females. These observations suggested that growth hormone participated in down-regulation of CYP2B9 expression in the males at least in part. Drugs or xenobiotics, i.e., phenobarbital, dexamethasone, DDT (1,1,1-trichloro-2,2-bis(*p*-chlorophenyl) ethane), are exogenous factors influencing the CYP2B expression. For example, phenobarbital and DDT induced both CYP2B9 and CYP2B10 while dexamethasone predominantly induced CYP2B10, but simultaneously suppressed CYP2B9. In conclusion, the factors affecting regulation of CYP2B expression should be thoroughly considered to eliminate confounding factors, leading to accurate and precise outcomes.

The study in recent years found that *CYP2B6* gene regulation in human influenced by the constitutive androstane receptor (CAR), which is a nuclear receptor that mediated the hepatic regulation and expression of a wide variety of genes involved in endobiotic and xenobiotic clearance.[19] CAR demonstrated a greater sexual dimorphic activity in females than males. Estrogens activated CAR and this may increase CAR activity in females relative to males.[20,21] Furthermore, androgens also inhibited CAR activity in mice[22], and this may reduce CAR activity in males relative to females. Altogether, these findings indicated that CAR may have greater activity in female mice and therefore help control and maintain basal and inductive expression of several sexually dimorphic CYPs.[23] Therefore, regulation mechanism of nuclear receptors mediated CYP2B expression is an interesting factor worth for further consideration.

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