Tissue Distribution, Gender-Divergent Expression, Ontogeny, and Chemical Induction of Multidrug Resistance Transporter Genes (Mdr1a, Mdr1b, Mdr2) in Mice

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ABSTRACT:

Multidrug resistance (Mdr) transporters are ATP-binding cassette transporters that efflux amphipathic cations from cells and protect tissues from xenobiotics. Unfortunately, Mdr transporters also efflux anticancer drugs from some tumor cells, resulting in multidrug resistance. There are two groups of Mdrs in mice: group I includes Mdr1a and Mdr1b that transport xenobiotics, whereas group II is Mdr2, a flipase that facilitates phospholipid excretion into bile. Little is known about the regulation of Mdr genes in vivo. The purpose of this study was to determine tissue distribution, gender differences, ontogeny, and chemical induction of Mdr transporters in mice. The mRNA of Mdr1a is highest in gastrointestinal tract, Mdr1b in ovary and placenta, and Mdr2 in liver. Both Mdr1a and Mdr1b in kidney show female-predominant expression patterns due to repression by androgens. The ontogeny of mouse Mdr1a in duodenum and brain as well as Mdr1b in brain, kidney, and liver all share a similar developmental pattern: low expression at birth, followed by a gradual increase to mature levels at approximately 30 days of age. In contrast, Mdr2 mRNA in liver is markedly up-regulated at birth, which returns to low levels by 5 days of age and then gradually increases to mature levels. None of the Mdrs in liver are readily inducible by any class of microsomal enzyme inducers. In conclusion, the three Mdr transporters in mice are expressed in a tissue-specific and age-dependent pattern, there are gender differences in expression, and Mdr transporters are inducible by only a few microsomal enzyme inducers.

Multidrug resistance transporters (AbcB1 family), also termed “P-glycoproteins,” were originally discovered in human (MDR1) and rodent (Mdr1a and Mdr1b) cancer cell lines, in which they were markedly up-regulated (Bradley et al., 1988). They function as efflux pumps with broad substrate profiles, including many chemotherapeutic drugs, hence the name “multidrug resistance.” In contrast, Mdr2 (AbcB2) is a flipase, which effluxes phospholipids from the inner to the outer membrane of the bile canaliculus (Smit et al., 1993). Phospholipids protect the biliary tree against bile acids, and they also form micelles with bile acids, which promote the absorption of lipid and lipid-soluble vitamins from the GI tract.

Little is known about the in vivo regulation of the mouse Mdr family of transporters. Tissue distribution and chemical induction of Mdr in rats have been characterized previously (Brady et al., 2002). However, because mice are becoming a more commonly used experimental model due to the availability of knockout animals, it is important to determine the expression pattern and regulation of the Mdr genes in mice.

Expression of Mdr transporters among tissues reflects the vulnerability of various tissues to Mdr substrates upon chemical exposure. The human MDR1 gene is expressed highly in the brush border of enterocytes, indicating its role in protecting the organism from some orally exposed xenobiotics (Thiebaut et al., 1987). Mouse Mdrs have been found in the intestinal epithelium, capillaries of brain and testis, adrenal gland, and ovaries (Buschman et al., 1992). However, a more specific approach is needed to probe for the different isoforms of the mouse Mdrs, and a quantitative comparison is desired to compare the expression of Mdr among various tissues. The branched DNA amplification technology has been shown to have high specificity, linearity, and efficiency (Hartley and Klaassen, 2000).

Gender- divergent expression of transporters and drug-metabolizing enzymes may result in differences in absorption, distribution, metabolism, and excretion of chemicals between males and females. Endocrine signals may be responsible for such differences. Sex hormones (5α-dihydroxytestosterone in males and 11β-estradiol in females) as well as efflux transporters may be in a competitive relationship with chemical substrates.

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ABBREVIATIONS: Mdr, multidrug resistance; GI, gastrointestinal; GH, growth hormone; MEI, microsomal enzyme inducer; AhR, aryl hydrocarbon receptor; CAR, constitutive androstane receptor; PXR, pregnane X receptor; PPAR, peroxisome proliferator-activated receptor; Nrfl2, nuclear factor erythroid 2-related factor 2; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; PCB126, polychlorinated biphenyl 126; lit-lit, growth hormone-releasing hormone receptor-deficiency; DHT, 5α-dihydroxytestosterone; E2, 11β-estradiol; GHRH, growth hormone-releasing hormone; BNF, β-naphthoflavone; PB, phenobarbital; TCPOBOP, 1,4-bis[2,3,5-trichlorophenyl]benzene; DAS, diallyl sulfide; PCN, pregnenolone-16α-carbonitrile; SPR, spironolactone; CLBF, clobifric acid; CPF, ciprofibrate; DEHP, diethylhexylphthalate; BHA, butylated hydroxyanisole; OPZ, oltipraz; bDNA, branched DNA; ANOVA, analysis of variance; FXR, farnesoid X receptor; FPGH, female-patterned growth hormone; GNX, gonadectomy; HX, hypophysectomy; MPGH, male-patterned growth hormone.
as secretion patterns of growth hormone are different between males and females. Male-patterned growth hormone consists of spikes in GH release, followed by troughs in which GH is virtually absent; female-patterned growth hormone is secreted in a consistent, pulsatile pattern, with relatively high troughs compared with those in males. Previous work in this laboratory has revealed gender-divergent expression of several mouse transporters in vivo, including some of the organic anion-transporting polypeptide uptake transporters and the multidrug resistance-associated protein efflux transporters, which have gender-divergent expression in liver, kidney, or both (Cheng et al., 2005; Maher et al., 2005). These gender differences were due to sex hormones, growth hormone secretory patterns, or both. Whether gender-divergent expression also occurs in the Mdr transporter family remains unknown; therefore, the expression patterns of mouse male and female Mdrs as well as the mechanisms of regulation will be determined in the present study.

Neonates are generally considered to be more susceptible to xenobiotics than adults, which in part might be due to less efficient drug metabolism and transport. In addition, infants have unique metabolic pathways compared with adults. For example, different isoforms of cytochrome P450 enzymes are found in human infants and adults (Tateishi et al., 1997). Therefore, in the present study, the ontogenic expression of total RNA was added to each well of a 96-well plate. Mdr mRNA was quantified by the branched DNA signal amplification assay (QuantMG bDNA signal amplification kit; Chappell, Texas, US) per the manufacturer’s protocol. RNA concentrations were quantified by ultraviolet absorbance at 260 nm.

**Materials and Methods**

**Chemicals.** 3,3’,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) was a gift from Dr. Karl Rozman (University of Kansas Medical Center, Kansas City, KS). Oltipraz was a gift from Dr. Steven Safe (Texas A&M University, College Station, TX). Polychlorinated biphenyl 126 (PCB126) was obtained from AccuStandard (New Haven, CT). All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO).

**Mice.** C57BL/6J mice were purchased from The Jackson Laboratory (Bar Harbor, ME) to study the tissue distribution, gender differences, ontogeny, and chemical induction of Mdrs. For the gender differences study, hypophysectomized (lit-lit) mice were also purchased from The Jackson Laboratory (Bar Harbor, ME) to study the tissue distribution, gender differences, ontogeny, and chemical induction of Mdrs. For the gender differences study, hypophysectomized (lit-lit) mice were also purchased from The Jackson Laboratory (Bar Harbor, ME) to study the tissue distribution, gender differences, ontogeny, and chemical induction of Mdrs.

**Branched DNA Signal Amplification Assay.** Mouse Mdr mRNA was quantified by the branched DNA signal amplification assay (QuantMG bDNA signal amplification kit; Chappell, Texas, US) per the manufacturer’s protocol. RNA concentrations were quantified by ultraviolet absorbance at 260 nm.

**Statistical Analysis.** Statistical differences between genders were determined by Student’s t test. Differences between multiple groups were analyzed by one-way ANOVA followed by Duncan’s post hoc test. Statistical significance was considered at \( p < 0.05 \).

**Results**

**Tissue Distribution of Mouse Mdrs.** The mRNA expression of the three Mdrs in 13 mouse tissues was quantified as shown in Fig. 1. The expression of Mdr1a mRNA (Fig. 1, top) is highest in the gastrointestinal tract, with levels increasing along the GI tract from stomach to colon [stomach 3%, duodenum (25%), jejunum (26%), ileum (43%), and colon (100%)]. Compared with the highest expression level in colon (100%), the expression of Mdr1a mRNA in other tissues, namely, liver (6%), kidney (5%), lung (9%), heart (9%), gonads (6%), and placenta (8%), was all less than 1/10 of that in colon; the brain Mdra1 mRNA is relatively higher (14%). Mdr1b mRNA is high in ovary and placenta, and it is expressed in kidney, heart, lung, and brain at intermediate levels. The highest level of expression of mouse Mdr1b mRNA is in ovary (100%), closely followed by that in placenta (64%). In comparison, testis has much

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lower expression of Mdr1b mRNA (20%). Moderate expression levels of Mdr1b mRNA were also observed in heart (45%), brain (36%), kidney (30%), and lung (30%). The expression of Mdr1b mRNA in the gastrointestinal tract is duodenum (13%), ileum (15%), jejunum (19%), stomach (19%), and colon (20%). Mdr2 mRNA is predominantly expressed in liver, but it is also expressed in heart (38.5%) and brain (23%).

Gender Differences in Mouse Mdrs. As shown in Fig. 1, gender-divergent expression of Mdrss was observed in the following tissues: Mdr1a and Mdr1b mRNA in kidney; Mdr1b and Mdr2 in lung, and Mdr1b in brain. In kidney, both Mdr1a and Mdr1b have a female-dominant expression pattern, as does Mdr1b and Mdr2 mRNA in lung. In contrast, the expression of Mdr1b in brain is male-dominant. Because kidney is a major site for drug elimination, the effects of hormones on kidney Mdr1a and Mdr1b are studied as shown in Figs. 2 and 3.

Effects of Gonadectomy, Hypophysectomy, and lit-lit on Mdr1a mRNA Expression. Mdr1a mRNA was 83% higher in female than in male kidney (Fig. 2, top). Gonadectomy increased Mdr1a mRNA in male mice (177% of control) and decreased Mdr1a mRNA in female mice (70% of control) and thus abolished the female-dominant expression pattern. Compared with gonadectomy alone, administration of DHT decreased Mdr1a mRNA in castrated mice (56% of gonadectomized male mice). Administration of E2 also decreased Mdr1a mRNA in castrated mice (66% of gonadectomized male mice), but it did not alter Mdr1a expression in ovariectomized mice. Together, these data suggest that male sex hormone inhibits the expression of male Mdr1a in kidney.

Hypophysectomy increased Mdr1a mRNA in male mouse kidney (200% of control), but it did not alter Mdr1a mRNA in female mice (Fig. 2, middle), and thus it abolished the female-dominant expression pattern. Compared with hypophysectomy alone, administration of male-patterned GH did not change Mdr1a mRNA expression in male or female mice. Administration of male-patterned growth hormone decreased Mdr1a mRNA in male (58% of hypophysectomized male mice), but it did not alter the female Mdr1a mRNA. In summary, male-patterned growth hormone inhibits Mdr1a expression in male kidney.

lit-lit increased Mdr1a mRNA in male mouse kidney (175% of control), but it decreased Mdr1a mRNA in female mouse kidney (67%
of control) (Fig. 2, bottom). Compared with vehicle-treated lit-lit mice, administration of male-patterned GH to the lit-lit mice decreased Mdr1a mRNA in male mice (71% of lit-lit male mice), but it did not change Mdr1a mRNA expression in female lit-lit mice. Administration of female-patterned GH did not alter either male or female Mdr1a mRNA expression.

Taken together, the female-predominant expression of Mdr1a mRNA in kidney of mice seems to be due to the inhibitory effect by androgens. The administration of male-patterned growth hormone decreased Mdr1a mRNA in kidneys of male mice, probably through elevating androgen levels.

**Effects of Gonadectomy, Hypophysectomy, and lit-lit on Mdr1b mRNA Expression.** Mdr1b mRNA was 117% higher in female than in male kidney (Fig. 3, top). Gonadectomy increased Mdr1b mRNA in castrated mice (270% of control), but it did not alter Mdr1b mRNA in ovariectomized mice. Compared with gonadectomy alone, administration of DHT decreased Mdr1b mRNA in kidneys of castrated mice (26% of gonadectomized male), but it did not alter Mdr1b mRNA in ovariectomized mice. Administration of E2 also decreased Mdr1b mRNA in kidneys of castrated mice (32% of gonadectomized female), but it did not alter it in ovariectomized mice. Together, these data indicate that androgen inhibits the expression of Mdr1b in kidneys.

Hypophysectomy increased Mdr1b mRNA in kidneys of both male mice (500% of control) and female mice (170% of control) (Fig. 3, middle). Administration of female-patterned growth hormone to hypophysectomized mice did not alter Mdr1b mRNA in male or female kidneys, but male-patterned GH decreased male Mdr1b mRNA in kidneys of hypophysectomized mice (35% of hypophysectomized male). Together, these data suggest that male-patterned growth hormone inhibits Mdr1b expression in mouse kidneys.

In contrast with control wild-type mice, Mdr1b mRNA was higher in male than female lit-lit mice (Fig. 3, bottom). Administration of male-patterned GH and female-patterned GH to lit-lit mice both decreased Mdr1b mRNA in kidneys of male (48 and 58% of lit-lit male, respectively), but not in female mice. Administration of male-patterned growth hormone also decreased Mdr1a and Mdr1b mRNA in males, probably through an indirect effect by elevating androgen levels. In summary, the female-predominant expression of both Mdr1a and Mdr1b in kidneys of mice seems to be due to inhibitory effect by androgen.

**Ontogeny of Mdr1a in Mouse Duodenum.** Duodenum is the first section of the small intestine and thus first exposed to xenobiotics and other chemicals in the intestine. In addition, duodenum is also the first section of the GI tract to come in contact with bile acids secreted from the liver. During development, duodenum is the first section of the intestine that senses changes in the diet from maternal milk to standard fiber-enriched chow. Therefore, duodenum was selected to examine the ontogeny of Mdr1a, even though its expression is not as high as in ileum and colon. The developmental pattern of Mdr1a in mouse duodenum is shown in Fig. 4 (top, first panel). Because no gender differences in Mdr1a were observed in duodenum, male and female tissues were combined at each age. There was minimal expression of Mdr1a in mouse duodenum prenatally. After birth, the Mdr1a mRNA expression increased in a time-dependent manner, which exhibited a peak (17-fold of day −2 levels) at 15 days of age, followed by a decrease thereafter.

**Ontogeny of Mdr1b in Mouse Kidney and Liver.** Mdr1b is crucial for renal excretion of some chemicals, because it transports xenobiotics from proximal tubular cells into the lumen. The developmental patterns of Mdr1b in male and female mouse kidney are shown in Fig. 4 (second panel). There was low expression of Mdr1b in mouse kidneys before 15 days of age in both male and female mice. After day 10, both male and female mouse Mdr1b mRNA expression in kidneys increased (approximately 150% of day −2 levels) until 30 days of age, when they reached their maximal expression levels, which are at least 2-fold higher than that at birth. After 30 days of age, there was a sharp decrease in male Mdr1b mRNA expression in kidney, whereas it remained high in female mice.

Mdr1b was low in adult liver as shown in Fig. 1. During development, Mdr1b in liver is consistently expressed at a low and steady level (Fig. 4, third panel).

The developmental pattern of Mdr2 in mouse liver is shown in Fig. 4 (bottom, fourth panel). Low expression of Mdr2 mRNA in mouse liver was noted 2 days before birth. At birth, the expression level increased approximately 3-fold, followed by a dramatic decrease to before birth levels by day 5 of age, which then gradually returned to

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**Fig. 3. Hormone effects on the expression of Mdr1b in kidney.** Top, kidneys were collected from naive mice (Cont), gonadectomized mice replaced with placebo (GNX), gonadectomized mice replaced with DHT (GNX+DHT), and gonadectomized mice treated with E2 (GNX+E2). Middle, kidneys from naive mice (Cont), hypophysectomized mice replaced with placebo (HX), hypophysectomized mice replaced with female-patterned growth hormone (HX+MPGH), and hypophysectomized mice replaced with male patterned growth hormone (HX+MPGH). Bottom, kidneys from naive mice (Cont), lit-lit mice replaced with placebo (lit-lit), lit-lit mice replaced with male patterned growth hormone (lit-lit+MPGH), and lit-lit mice replaced with female-patterned growth hormone (lit-lit+FPGH). Total RNA was isolated and Mdr1b mRNA levels were quantified by qPCR assay. Asterisks (‡) indicate statistically significant differences between male and female mice compared by a Student’s t test (p < 0.05). Single daggers (†) indicate statistically significant differences compared with naive mice. Double daggers (‡) indicate statistically significant differences compared with the placebo-treated surgery groups (GNX, HX, or lit-lit) (ANOVA, followed by Duncan’s post hoc test, p < 0.05). RLU, relative light units.
adult levels (approximately 3-fold of day −2 levels) by 1 month of age.

**Ontogeny of Mdr1a and 1b in Mouse Brain.** The blood-brain barrier is important to protect the brain from excessive chemical exposure. Mdr transporters are expressed in the endothelial cells of the brain and thus serve a protective role for the brain. The developmental patterns of Mdr1a and 1b in male and female mouse brain are shown in Fig. 5. Mdr1a and 1b mRNA in male and female mice share similar developmental patterns. Minimal expression of Mdr was observed 2 days before as well as the first 10 days of age in both male and female mice. The Mdr1a and 1b mRNA expression levels increased thereafter in both male and female mice, and they reached their mature levels at 30 days of age. Between birth and 6 weeks of age, there is approximately a 200% increase in Mdr1a and only a 50% increase in Mdr1b.

**Chemical Induction of Mdrs.** Fifteen chemicals, which fall into five groups of transcription factor activators (also known as microsomal enzyme inducers), were used at doses shown in Table 1. Because the inducers are not necessarily specific for one transcription factor, for example, BNF is an AhR activator but can be metabolized to an Nrf2 activator (Huang et al., 2000; Wang et al., 2006), three inducers were used per transcription factor, to generate more confidence that the induction is probably mediated by a certain transcription factor. The chemical induction of the three Mdrs was studied in liver, brain, and duodenum. No statistical difference in mRNA expression was observed among the three vehicle-treated groups (corn oil by intraperitoneal, corn oil by gavage, and saline intraperitoneal route). Therefore, these three control groups were averaged as a single vehicle control group.

The effect of microsomal enzyme inducers on the Mdr mRNAs in mouse liver is shown in Fig. 6. Overall, none of the Mdrs were induced by any entire class of microsomal enzyme inducer. Only a few ligands induced Mdrs, namely, the PPARα ligand DEHP induced Mdr1a (187% of control), the CAR ligand TCOBP in induced Mdr1b (154% of control), and the PPARα ligands CFBP and DEHP induced Mdr2 in liver (192 and 160% of control, respectively).

The effect of microsomal enzyme inducers on the Mdr mRNAs in mouse brain is shown in Fig. 7. The CAR ligand DAS induced both Mdr1a (267% of control) and Mdr1b mRNA (150% of control), the CAR ligand TCOBP induced Mdr1b (200% of control); and Mdr2, 150% of control). Another PXR ligand PCN induced all three Mdrs (Mdr1a, 220% of control; Mdr1b 200% of control; and Mdr2, 150% of control). Another PXR ligand SPR, induced Mdr2 in brain (200%).

The induction of Mdr mRNAs in mouse duodenum is shown in Fig. 8. Neither Mdr1b nor Mdr2 seemed to be inducible in the duodenum by any MEI. Mdr1a mRNA was induced by the PXR ligand PCN (200%), by PPARα ligands CFβ (200%) and DEHP (300%), and by Nrf2 activators BHA (200%) and OPZ (225%) but not by any entire class of MEI.

**Discussion**

Mdr1 transporters are key molecules in determining not only the resistance of cancer cells against chemotherapeutic drugs but also the disposition of a variety of drugs in intestine and other tissues. The mouse tissue with the highest Mdr1a mRNA is colon, which probably...
indicates its high capability to excrete toxicants. The human ortholog for mouse Mdr1a is MDR1, which has been found in human colon carcinoma cell lines, and MDR1 is inducible by several herbal medicinal products and food supplements in human cancer cell lines (Brandin et al., 2007); therefore, it probably prevents the absorption of xenobiotics, enhances the excretion of xenobiotics, or both. However, the high expression of Mdr1a mRNA in colon may also contribute to colon cancer cell resistance to chemotherapeutic drugs. Many cancer chemotherapeutic drugs are Mdr1/MDR1 substrates. This was demonstrated by genetically engineered mice without the Mdr1 genes. For example, Mdr1a-null mice excrete less vinblastine, a cancer chemotherapeutic drug, into the intestinal lumen (van Asperen et al., 2000). The Mdr2 transporter is unique, because it is a phospholipid flipase rather than a xenobiotic transporter. It is predominantly expressed in liver, and it transfers phospholipid from the inner to outer canalicular membrane to protect the biliary tree from bile acids as well as forming biliary emulsions. The Mdr1b transporter, which has similar functions as Mdr1a, has a very different tissue distribution pattern in mice (Fig. 1). Mdr1b mRNA is most abundantly expressed in ovary and placenta, which indicates it probably has a more important protective role against xenobiotics in females. It has been shown that several sex hormones are substrates for human MDR1 in vitro (Ueda et al., 1992). The highly expressed Mdr1b in mouse ovary may protect the tissue against excessive hormone accumulation. Placental Mdr1b is considered a protective mechanism against xenobiotic insults to the fetus (Behravan and Piquette-Miller, 2007). The expression of both Mdr1a and 1b mRNA is low in liver compared with the GI tract, indicating that the hepatic excretion of xenobiotics into bile canaliculi is probably more dependent on other apical efflux transporters. The Mdr2 transporter is unique, because it is a phospholipid flipase rather than a xenobiotic transporter. It is predominantly expressed in liver, and it transfers phospholipid from the inner to outer canalicular membrane to protect the biliary tree from bile acids as well as forming biliary emulsions. The Mdr1b transporter, which has similar functions as Mdr1a, has a very different tissue distribution pattern in mice (Fig. 1). Mdr1b mRNA is most abundantly expressed in ovary and placenta, which indicates it probably has a more important protective role against xenobiotics in females. It has been shown that several sex hormones are substrates for human MDR1 in vitro (Ueda et al., 1992). The highly expressed Mdr1b in mouse ovary may protect the tissue against excessive hormone accumulation. Placental Mdr1b is considered a protective mechanism against xenobiotic insults to the fetus (Behravan and Piquette-Miller, 2007). The expression of both Mdr1a and 1b mRNA is low in liver compared with the GI tract, indicating that the hepatic excretion of xenobiotics into bile canaliculi is probably more dependent on other apical efflux transporters. The Mdr2 transporter is unique, because it is a phospholipid flipase rather than a xenobiotic transporter. It is predominantly expressed in liver, and it transfers phospholipid from the inner to outer canalicular membrane to protect the biliary tree from bile acids as well as forming biliary emulsions.
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versely, it was shown in this laboratory that the xenobiotics uptake
familial intrahepatic cholestasis (Maisonnette et al., 2005) and intrahe-
patic cholestasis of pregnancy (Dixon et al., 2000).

Gender-divergent expression of efflux transporters indicates differ-
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transporters, organic anion-transferring polypeptides 1a1 and 3a1, are
male-predominant (Cheng et al., 2006). This suggests that female
mice may accumulate less of the drug in liver than male mice.

The female-predominant pattern of Mdr1a and 1b expression is due
to the repression by androgens in mouse kidneys (Figs. 2 and 3). In
addition, exogenous estrogen also decreased Mdr1a and 1b mRNA
expression in male kidneys. It has been demonstrated that P-glyco-
protein levels are inducible by several types of female sex hormones
(estrone, estriol, and ethynyl estradiol) in Madin-Darby canine kidney
cells (Kim and Benet, 2004). The difference between the previous
studies and the present finding could be due to different models and
study approaches. First, it should be remembered that a cell line often
does not replicate the in vivo situation. Second, administration of exog-
enous estradiol may activate other nuclear receptors in an estrogen-
dependent way, which may exert inhibitory effects on the expression of
Mdr1s.

Adult brain is a privileged site due to protection by the blood-brain
barrier. The expression of Mdr1a and 1b on the blood-brain barrier is
important in protecting against xenobiotic insults, by decreasing the
penetration of drugs into brain. It has been shown that Mdr1a-null mice have higher brain concentrations of ivermectin, vinblastine,
digoxin, and cyclosporine A after their administration (Schinkel et al.,
1994). Disruption of both Mdr1a and 1b in mice results in enhanced
penetration of steroid hormones, such as corticosterone, cortisol, aldoste-
ronone, and progesterone into brain (Uhr et al., 2002). The expression of
both Mdr1a and 1b mRNA is low in the pre- and neonatal brain and other
organs. The low expression of Mdr1s in brain correlates with the high
vulnerability to xenobiotics in newborns, which results in a challenge in
treating newborn diseases without significant side effects to the brain. In
contrast, the expression of Mdr2 in liver was high at birth, which even
exceeded adult levels. Mdr2 pumps phospholipids into bile, which sub-
sequently aids in digestion and absorption of lipophilic compounds.
Therefore, enhanced expression of Mdr2 at birth may benefit the new-
borns in nutrient acquisition, including fat from milk, and lipophilic
vitamins (A, D, E, and K) that are essential for development. Neonatal
up-regulation of the mRNAs of several nuclear receptors in liver was
observed, including CAR, PXR, FXR, PPAR, and hepatocyte nuclear
factor 4α, by the present laboratory (data not shown). Within 10 kilobases
of the promoter region of Mdr2, apparent binding sites for PXR, FXR,
CAR, hepatocyte nuclear factor 4α, and PPAR have been identified
(NHRscan software; Sandelin and Wasserman, 2004). It is possible that
some of these nuclear receptors may mediate the expression of Mdr2
during development. Our preliminary data indicate that the induction
of Mdr2 in newborns is abolished in FXR-null mice, indicating the neonatal
surge of Mdr2 is FXR-dependent.

The microsomal enzyme inducers used in the present study are
common chemicals found in the environment or as pharmaceutical
drugs. For example, TCDD is found in burnt trash; phenobarbital is
used for treating seizures; and ethoxyquin, a quinoline-based antiox-
idant, is used as a food preservative. These chemicals at the dosages
used in this study were found to up-regulate cytochrome P450 en-
zymes in rats and mice (Hartley and Klaassen, 2000; Cheng et al.,
2005). However, the present study indicates that only a few chemicals
induce Mdr transporters. It has been demonstrated that dosing rats
with PXR agonists PCN and dexamethasone both increased P-glyco-
protein expression in liver plasma membranes and in brain
capillaries (Bauer et al., 2004). In the present study, PCN adminis-
tration increased Mdr1a and 1b mRNA expression in rat brain, but it
did not alter the expression of any of the three Mdrs in liver. These
different observations on the chemical induction of Mdr2 could be due
to differences in species (rats versus mice), doses (the present study
used higher doses of PCN and dexamethasone than the previous study),
or detection methods (the present study detected mRNA, whereas the
previous study detected protein, and the increase in Mdr protein but not
mRNA might indicate post-transcriptional modifications of Mdr2). In
addition, it should be noted that dexamethasone is also a potent ligand for
the glucocorticoid receptor. Therefore, the induction of Mdr2 by dexa-
methasone in liver could also involve activation of PXR-independent
pathways. Down-regulation of liver Mdr2 occurred in mice treated with
PB and DAS. It has been shown that CLFB in the diet induces mouse
Mdr2 (Chianale et al., 1996). This was not observed in the present study,
which may due to strain differences, or to route of administration.
In conclusion, the present study examined the tissue distribution of
Mdr mRNAs in mouse and reveals gender-divergent expression of
Mdrs, demonstrates the effect of hormones on their expression, de-
termines the ontogenic expression patterns of Mdr mRNAs, and tests
the inducibility of Mdrs by microsomal enzyme inducers.
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References


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