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Altered Expression of Hepatic Cytochromes P-450 in Mice Deficient in One or More *mdr1* Genes

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ABSTRACT

We hypothesized that the drug efflux protein P-glycoprotein (Pgp), the product of the multidrug resistance gene *MDR1,* might influence hepatic expression of CYP3A or other cytochromes P-450 (P-450s) because Pgp can transport endogenous regulators of these cytochromes. We began with variants of a CF-1 mouse strain containing a defective *mdr1a* gene that is inherited in a Mendelian fashion. The amount of CYP3A protein in liver was inversely related to the gene dose of the normal *mdr1a* allele in these mice. *mdr1a* knockout mice of either mixed (FVB × 129/Ola) or pure FVB genetic background and housed in Amsterdam display an increased expression of CYP2B and CYP3A proteins. However, because *mdr1a* ablation causes a compensatory increase in hepatic *mdr1b* (which can efflux intracellular glucocorticoids), we reasoned that *mdr1b* might mask the overall effect of *mdr1a* absence on P-450 gene expression. Targeted inactivation of the *mdr1b* gene increased P-450 expression, but the effect was modest compared with *mdr1a* ablation. Mice nullizygous for both *mdr1a* and *mdr1b*-type Pgps and kept in Amsterdam had dramatically increased levels of CYP3A protein as well as other P-450s examined and of the electron donor P-450 reductase. Consistent with the protein results, CYP3A catalytic activity measured as midazolam 1′- and 4-hydroxylation in liver microsomes from these knockout mice revealed a rank order of activities with *mdr1a/1b* > *mdr1a* > *mdr1b* > (+/+) mice. In contrast to results in mice housed in Amsterdam, in the genetically identical *mdr1a* or *mdr1a/1b* (−/−) male mice housed in the United States, hepatic P-450 expression was unaffected by *mdr1* genotype or actually showed a slight decrease in *mdr1a* (−/−) mice. These results provide a revealing picture of *mdr1*-type Pgp as an upstream regulator of hepatic P-450 expression, and demonstrate that these pharmacologically relevant phenotypes in knockout mice depend not only on the genetic make-up of the mice but also on the environment.

Interindividual differences in expression of hepatic cytochromes P-450 (P-450s) that catalyze the oxidative metabolism of many drugs account for much of the human variation in drug elimination. This variation extends to all of the human liver P-450s, including CYP3A, CYP2C, CYP1A, CYP2D, and CYP2E. Because differences in P-450 expression can affect both drug efficacy and drug toxicity (and therefore therapeutic outcome), the factors influencing variation in expression of hepatic P-450s are under intense investigation.

Hormones are recognized regulators of many of the P-450s. Glucocorticoid hormones, including the endogenous rodent glucocorticoid corticosterone, are up-regulators of CYP3A expression in many species, including mice (Wrighton et al., 1985) and humans (Watkins et al., 1985). Recently, two groups (Blumberg et al., 1998; Kliewer et al., 1998) described additional hormonal regulators of CYP3A after their identification of the pregnane X receptor (PXR), a nuclear hormone receptor that mediates steroid transcriptional induction of CYP3A. These hormones included pregnenolones, progesterone, cortisol, cortisone, corticosterone, dihydrotestosterone, and estradiol. Importantly, Blumberg et al. identified putative binding motifs for PXR in the 5′-flanking sequences of many other P-450 genes, including members of the CYP2A, CYP2C, and CYP2E families and NADPH P-450 reductase (P-450 reductase)—not surprising, given that many of these P-450s are hormonally regulated. Clearly, factors that modulate steroid response could influence P-450 gene expression.

There is a growing awareness that intracellular action of steroids can be regulated by ATP-binding cassette transporters. The ATP-binding cassette transporter LEM-1, which transports glucocorticoids in yeast, modulates the biological potency of steroid hormones, specifically decreasing the in-

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ABBREVIATIONS: P-450, cytochrome P-450; PXR, pregnane X receptor; P-450 reductase, NADPH cytochrome P-450 reductase; Pgp, P-glycoprotein, the product of the human *MDR1* and mouse *mdr1a* and *mdr1b* genes; *MDR1*, multidrug resistance gene.

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tracellular concentration of glucocorticoids and, hence, glucocorticoid receptor activation (Kralli et al., 1995). Similarly, another ATP-binding cassette transporter has been shown to modulate intracellular thyroid hormone content and thyroid hormone receptor activation (Ribeiro et al., 1996). In rodents, the product of the *mdr1a* and *mdr1b* genes, P-glycoprotein (Pgp), seems to be a relevant transporter of steroid hormones (Barnes et al., 1996; Meijer et al., 1998). In fact, there is extensive overlap between drugs and steroids that are substrates for Pgp and drugs and steroids that are modulators and/or substrates for at least CYP3A (Schuetz et al., 1996a). We previously demonstrated that Pgp could influence the intracellular concentration of a CYP3A inducer, rifampicin, and thus influence a pharmacological action of this drug in the cell, namely the magnitude of CYP3A induction (Schuetz et al., 1996b). Because Pgp also transports steroids, and potentially other unidentified physiological modulators of P-450s, we hypothesized that Pgp might further regulate the expression of CYP3A and other P-450s. Indeed, we have reported a trend toward an inverse relationship between Pgp and CYP3A in human liver in vivo (Schuetz et al., 1995). Assessing the influence of Pgp on CYP3A expression in vivo is complicated by the fact that, although humans express a single *MDR1* gene, rodents share the function of *mdr1* between two highly homologous *mdr1*-type genes, *mdr1a* and *mdr1b*, and both genes are expressed in liver. Consequently, to determine whether there is a role of Pgp in vivo in P-450 expression, and to determine the extent to which *mdr1a* and *mdr1b* mediate unique or redundant effects on P-450s, we utilized mice lacking either singly or simultaneously the *mdr1a* and *mdr1b* genes. The results from this investigation support a model in which Pgp modulates the expression of not just CYP3A but many forms of cytochrome P-450, as well as P-450 reductase.

Materials and Methods

Mice

Mice Housed in Amsterdam. Mice (all 11–12 weeks old) were maintained at six to eight mice/cage with commercial "Woody-Clean type 8/15" bedding (BMI, Helmond, The Netherlands). The bedding is routinely checked by the manufacturer for a range of contaminants including chlorinated hydrocarbons and phosphor-containing pesticides. Animals had free access to commercial chow (AM-II; Hope Farms, Woerden, the Netherlands) and acidified water in the Netherlands Cancer Institute Animal Building. Male and female *mdr1a* (+/+) and (−/−) mice (FVB × 129/Ola) (Schinkel et al., 1994) and *mdr1a*, *mdr1b*, and *mdr1a/1b* (+/+) and (−/−) mice (FVB) (Schinkel et al., 1997) have been described previously.

Mice Housed in the United States. CF1 male mice (18–20 g) were obtained from Charles River Breeding Lab (Wilmington, MA). *Mdr1a* (FVB) and *mdr1a/1b* (FVB) (+/+) and (−/−) mice were obtained from Taconic Farms (Germantown, NY). Except for male *mdr1a/1b* (+/+) and (−/−) mice from Taconic (analyzed the day of arrival), all mice were housed in the St. Jude Children's Research Hospital animal facility for a minimum quarantine of 3 weeks before use. Male mice ranged in age from 7 to 12 weeks, but were all age-matched within experiments. Mice were maintained at 6 to 8 mice/cage with commercial bedding [("Beta-Chip," a nonacidic, mill-run sawdust of poplar, beech, or maple that is sifted, dried, and heat-treated to 180°F (Northeastern Products Corp., Columbia, KY)]. Mice had free access to commercial chow #5010 (Purina Mills, Indianapolis, IN; detailed in the reference manual at http://www.labdiet.com) and nonacidified, reverse-osmosis pure water. The dietary formulations are described in Table 1.

Determining *mdr1a* Genotype of the CF1 Mice

mdr1a genotype of all CF1 littermates was determined by Southern blotting of tail DNAs for the previously identified restriction

TABLE 1
Diets in the U.S. and Amsterdam

Chemical Composition	U.S.	Amsterdam
Nutrients		
Protein, %	23.5	27.2
Amino acids, %		
Lysine	1.4	1.38
Cystine	0.34	0.44
Methionine	0.49	0.47
Threonine	0.94	0.98
Tryptophan	0.29	0.25
Arginine	1.4	1.79
Phenylalanine	1.08	1.07
Tyrosine	0.64	0.75
Leucine	1.87	1.85
Isoleucine	1.24	1.01
Histidine	0.58	0.55
Valine	1.22	1.41
Glycine	1.2	N/A
Serine	1.23	N/A
Aspartic Acid	2.68	N/A
Glutamic Acid	5.02	N/A
Alanine	1.49	N/A
Proline	1.73	N/A
Taurine	0.03	N/A
Fat (ether extract), %	5.1	N/A
Fat (acid hydrolysis), %	6.2	N/A
Fat, %	N/A	7.2
Crude Fiber	3.9	4.1
Nitrogen-free extract	50.3	56.1
Gross energy, kcal/g	4.06	4.65
Minerals, %		
Ash, %	7.2	N/A
Calcium	1	0.84
Phosphorous	0.67	0.58
Sodium	0.28	0.3
Potassium	0.92	0.88
Magnesium	0.22	0.23
Chlorine	0.39	0.35
Vitamins		
Vitamin A, IU/g	44.1	17.5
Vitamin D, IU/g	4.4	2.0
Vitamin E	66.1 I.U./kg	109 mg/kg
Thamin	90.7 ppm	22.5 mg/kg
Riboflavin, mg/kg	8.0	14.4
Niacin, mg/kg available	128	36.4
Pantothenic acid, mg/kg	25	32.0
Folic acid, mg/kg	6.0	4.6
Pyridoxine (B-6) mg/kg	17.0	17.2
Choline	2200 ppm	2080 mg/kg
Vitamin B12, mcg/kg	33	55
Biotin (H) μg/kg	350	±300
Essential trace elements, mg/kg		
Iron	240	190.0
Manganese	115	70.4
Zinc	124	87.0
Cobalt	0.44	0.2
Iodine	1.2	0.35
Selenium	0.31	0.29
Tin	N/A	1.0
Chromium	2.0	1.5
Nickel	N/A	2.0
Vanadium	N/A	0.134

N/A, information not available.

fragment length polymorphism with the enzyme *Pst*I (Umbenhauer et al., 1997).

Dexamethasone Distribution Study

Male CF1 mice were dosed with the following formulations such that 100 μl of drug was administered per 10 g of body weight. Radiolabeled dexamethasone was added to the drug stocks. Dexamethasone (10 mg/kg) with 1 μCi [^{3}H]dexamethasone/10 g body weight in corn oil was administered orally by gastric gavage 5 h before sacrifice. Animals were anesthetized at sacrifice with metofane and blood was obtained by cardiac puncture into heparinized tubes. Tissues were removed and flash frozen. Tissues were weighed and homogenized in 4% (w/v) bovine serum albumin, and 200-μl aliquots were analyzed by liquid scintillation counting. The statistical significance of differences between total radioactivity levels in tissues of CF1 with *mdr1a* genotype (+/+) versus (+/−) and (−/−) mice was determined using the Student's unpaired two-tailed *t* test.

Immunoblot Analysis. Mouse liver microsomes were prepared (Watkins et al., 1985) and 10 or 20 μg of protein was separated on 10% slab polyacrylamide gels and immunoblotted using the following antibodies: monoclonal anti-CYP3A1 Ig8 (Hostetler et al., 1987), monoclonal anti-CYP3A4 K03 (Beaune et al., 1985), or polyclonal goat anti-CYP3A1 antibody (Hostetler et al., 1987). Monoclonal antibodies against rat CYP1A (CD2,3,5) and rat CYP2B (be26) were obtained from Dr. Paul Thomas (Rutgers University, Piscataway, NJ). Rabbit anti-rat CYP4A (Dr. Richard Okita, Washington State University, Pullman, WA), anti-rat P-450 reductase (Dr. Ken Thummel, University of Washington, Seattle, WA), anti-mouse liver testosterone 15α-hydroxylase (CYP2A4), and anti-mouse liver testosterone 16α-hydroxylase (CYP2D9) (Dr. M. Negishi, National Institute of Environmental Health Sciences, Research Triangle Park, NC) were generously provided by the indicated investigators. All primary antibodies were followed by appropriate secondary antibodies coupled with peroxidase and developed with the enhanced chemiluminescence detection system (Amersham, Arlington Heights, IL). P-450 levels were immunoquantified by comparing the densitometric values obtained for microsomal samples of individual *mdr1* (+/+) with (−/−) mouse livers analyzed on the same blots.

Identification of Mouse Liver CYP3A11 Protein on Immunoblots Developed with Anti-rat CYP3A1 Antibodies

We have determined, by SDS-polyacrylamide gel electrophoresis analysis (10% slab gels) of mouse liver microsomes, that the protein of higher molecular mass that immunoreacts with anti-CYP3A1 (monoclonal and polyclonal IgGs) is CYP3A11, because 1) this band comigrates with purified CYP3A11 (Bornheim and Correia, 1990) [generously provided by Dr. Lester Bornheim (Univ. of California, San Francisco, CA)] and 2) mixing experiments of CYP3A11 together with the mouse liver microsomes revealed that CYP3A11 comigrated with the upper band (results not shown). Purified CYP3A11 failed to immunoreact with anti-CYP3A4 IgG (results not shown).

Midazolam Hydroxylation

4-Hydroxymidazolam and 1′-hydroxymidazolam were assayed as described previously (Schuetz et al., 1996b). Briefly, mouse liver microsomes (0.1 mg/incubate) were preincubated for 3 min with midazolam at 37°C. The reaction was initiated by addition of one-tenth volume of an NADPH-generating system (10 U/ml isocitrate dehydrogenase, 50 mM isocitrate, 10 mM sodium NADP, and 50 mM magnesium chloride). Samples were incubated at 37°C for 10 min and the reaction was stopped by addition of 100 μl of cold methanol. Proteins were removed by centrifugation at 10,000*g*, and 50 μl of supernatant was injected directly on the high-performance liquid chromatography system. Enzyme activities were expressed as nanomoles of product per milligram of microsomal protein per hour.

RNA Isolation and Northern Blot Analysis

Total RNA was isolated from mouse livers (Schuetz et al., 1988) and analyzed by Northern blot with an albumin cDNA. Blots were autoradiographed and band intensities were quantified by densitometry.

Results

CF1 Mice from Charles River. To test the hypothesis that *mdr1* could influence P-450 gene expression, we used a subpopulation of CF1 mice (from Charles River) that are deficient in *mdr1a* but still express *mdr1b* (Umbenhauer et al., 1997) and that have characteristics similar to those described in mice with targeted disruption of the *mdr1a* gene (Schinkel et al., 1994; Umbenhauer et al., 1997). We took this opportunity to simultaneously determine whether a prototypical CYP3A up-regulator (dexamethasone), which is also an *mdr1*-type Pgp substrate, would induce CYP3A to the same extent in CF1 mice with different *mdr1a* genotypes. [^{3}H]Dexamethasone was administered orally to 24 CF1 mice and 5 h later the radioactivity was measured in plasma, liver, and brain. There was no significant difference in [^{3}H]dexamethasone plasma or liver levels among genotypes, but there was a statistically higher concentration of [^{3}H]dexamethasone in the brains (and in the brain/plasma ratio) of the *mdr1a* (−/−) genotype compared with the (+/+) genotype (Table 2). This result is consistent with a role for Pgp in the brain penetration of dexamethasone (Schinkel et al., 1995).

Next we examined the expression of CYP3A proteins among the 22 untreated (control) CF1 mice and 23 CF1 mice treated orally with 10 mg/kg dexamethasone for 5 h. Representative immunoblots showed that there was substantial heterogeneity in CYP3A expression among each of the genotypes (Fig. 1A), which is probably caused by the outbred genetic background of the CF1 mice. Nevertheless, CYP3A expression was inversely related to the gene dose of the *mdr1a* (+) allele (Fig. 1B), although this relationship does not reach statistical significance. Dexamethasone given at high doses and for long durations is an efficacious inducer of mouse liver CYP3A (Wrighton et al., 1985). However, to minimize the influence of metabolism to dexamethasone dis-

TABLE 2

Tissue levels of total radioactivity in CF1 mice with different *mdr1a* genotypes 5 h after oral gavage of [^{3}H]dexamethasone (10 mg/kg)

Data are shown as mean ± S.D. Statistical difference in radioactivity in either (+/−) or (−/−) were compared with (+/+) mice by a two-sided Student's *t* test with $P < .05$ as the limit of significance.

Genotype	Plasma	Brain	Brain/Plasma	Liver	Liver/Plasma
(+/+) (n = 6)	1,361.0 ± 157.5	204.7 ± 80.6	0.156 ± 0.069	14,511 ± 2,484	10.75 ± 2.02
(+/−) (n = 11)	1,428.7 ± 229.5	211.8 ± 28.6	0.152 ± 0.03	16,235 ± 3,484	11.46 ± 2.24
(−/−) (n = 7)	1,332.6 ± 286.4	341.7 ± 96.1*	0.256 ± 0.045*	15,256 ± 3,210	11.59 ± 1.82

* $P < .05$.

position in analysis of CF1 mice without *mdr1a*, we chose a low oral dose and short duration of dexamethasone. Unfortunately, hepatic CYP3A was not induced by this dose, vehicle, or duration of dexamethasone treatment in any of the CF1 mice (Fig. 1B), which is also consistent with the finding of no significant difference in total liver radioactivity between genotypes.

***mdr1a* (−/−) and (+/+) Mice (FVB × 129/Ola) Housed in Amsterdam.** We next determined whether *mdr1* could influence P-450 gene expression in age- and gender-matched mice with defined, targeted disruption of the *mdr1a* gene. In these first studies, mice were housed in Amsterdam and were heterozygote outcrosses from FVB and 129/Ola resulting in heterogeneity in the genetic background among the progeny. Compared with the *mdr1a* (+/+) mice, the *mdr1a* (−/−) mice displayed a 2- to 3-fold increase in the expression of CYP3A11 and the other anti-CYP3A1 immunoreactive protein, and this phenotype was independent of gender (Fig. 2). A measure of CYP3A activity in these same microsomes, however, revealed no difference between *mdr1a* (−/−) and (+/+) mice in either 4-hydroxymidazolam formation rates (34.16 ± 8.7 and 38.58 ± 6.27 nmol/mg/h, respectively) or 1′-hydroxymidazolam formation rates (47.84 ± 8.8 and 47.16 ± 7.2). The most dramatic effect of *mdr1a* ablation was observed in CYP2B proteins in female liver, which were 3.3 times greater in amount in *mdr1a* (−/−) than (+/+) mice (Fig. 2; Table 3). However, one of the (+/+) female mice exhibited a similarly elevated expression of CYP2B proteins. If this outlier (+/+) mouse is excluded from the comparison, we find CYP2B protein is 12-fold greater in the *mdr1a* (−/−) compared with (+/+) mice. Because CYP2B proteins are regulated by estradiol and progesterone (Nemoto and Sakurai, 1995), hormones that are not static in female mice, it is a distinct possibility that the single female *mdr1a* (+/+) mouse with elevated CYP2B was either pseudopregnant or was singularly experiencing some other change in sex hormones compared with the other female *mdr1a* (+/+) mice, or that CYP2B was influenced by the mixed genetic background of the mice. The influence of *mdr1* on CYP2B was gender specific because no difference in CYP2B expression was observed among the male *mdr1a* (−/−) and (+/+) mice (Fig. 2). There was no significant difference in CYP1A gene expression among these mice.

***mdr1a* and *mdr1b* (−/−) and (+/+) Mice (FVB) Housed in Amsterdam.** To avoid complications resulting from a mixed genetic background and to address the possible role of *mdr1b* [which is overexpressed in *mdr1a* knockout mice (Schinkel et al., 1994)] in P-450 gene expression, we used male *mdr1a* and *mdr1b* knockout mice in a pure FVB genetic background when these became available, and housed them in Amsterdam. An analysis of hepatic P-450 expression in male mice revealed a striking increase in CYP3A expression in the *mdr1a* (−/−) mice and a substantial but lesser increase in *mdr1b* (−/−) mice (Fig. 3; Table 3). In addition to CYP3A, we examined the expression of several other hepatic P-450s and P-450 reductase involved in steroid and drug metabolism by immunoblot analysis. Surprisingly, there was a significant increase in the expression of each and every P-450 examined and P-450 reductase in *mdr1a* (−/−) mice, although a lesser increase was observed in *mdr1b* (−/−) mice (Fig. 3). However, the magnitude of effect of *mdr1* genotype on expression of each isoform of P-450 varied (Table 3).

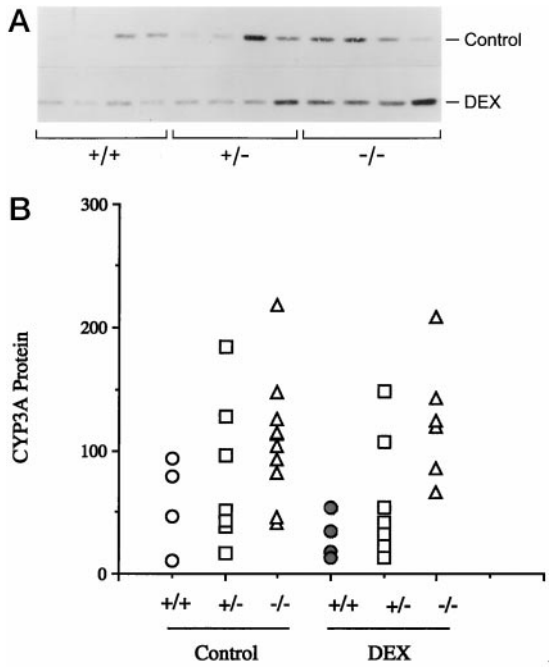

Fig. 1. A, hepatic expression of CYP3A in male CF1 mice. Ten micrograms of microsomal protein from the livers of individual untreated (control) or dexamethasone-treated male CF1 [either (+/+), (+/−), or (−/−)] from Charles River (Wilmington, MA) were analyzed by immunoblot using anti-CYP3A1 monoclonal antibody and enhanced chemiluminescence detection. B, relationship between CYP3A phenotype and *mdr1a* genotype in male CF1 mice. Ten micrograms of liver microsomal protein were analyzed by immunoblot with anti-CYP3A1 IgG. CYP3A protein was quantified in each sample by comparing the densitometric value obtained for microsomal samples of each mouse liver relative to the densitometric value obtained for an internal standard run in duplicate on each blot, arbitrarily set at 100%. Average values ± S.D. for untreated control mouse livers: (+/+), 48.1 ± 38.4, $n = 5$; (+/−), 80 ± 59.6, $n = 7$; and (−/−), 108.5 ± 50.9, $n = 10$. Average values ± S.D. for dexamethasone-treated mouse livers: (+/+), 24.49 ± 16.4, $n = 6$; (+/−), 57.86 ± 46.3, $n = 8$; (−/−), 107.5 ± 30.7, $n = 5$. This analysis was performed on at least two separate filters to account for blot-to-blot variation.

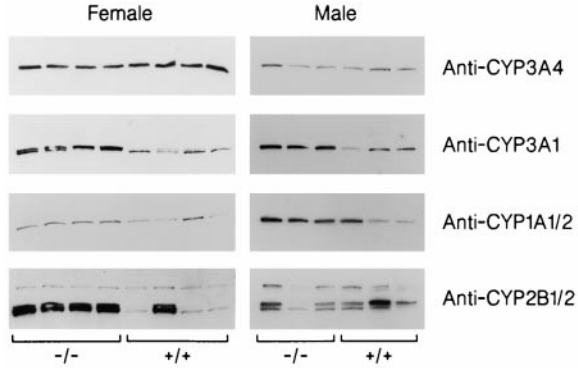

Fig. 2. Expression of P-450 proteins in the livers of male and female *mdr1a* (+/+) and (−/−) (FVB × 129/OLA) mice housed in Amsterdam. Ten micrograms of microsomal protein from the livers of individual *mdr1a* (+/+) and (−/−) female and male mice 11 to 13 weeks of age were analyzed by immunoblot using either monoclonal antibodies against CYP1A1/2, CYP2B, CYP3A1, or CYP3A4.

***mdr1a/1b* (−/−) and (+/+) Mice (FVB) Housed in Amsterdam.** Analysis of the livers of male and female *mdr1a/1b* double-knockout mice from Amsterdam confirmed and extended the conclusion that *mdr1a* and *mdr1b* regulate hepatic P-450s. P-450 expression was significantly higher in the *mdr1a/1b* (−/−) mice (Fig. 4), although the extent to which each P-450 was affected varied. For example, in female mice, CYP2B was 11.5-fold higher in (−/−) mice than in (+/+), whereas CYP3A was 4.5-fold higher in the same (−/−) mice. There was also a 2.7-fold increase in the expression of the electron donor P-450 reductase in the male *mdr1a/1b* (−/−) mice. Although the magnitude of effect of *mdr1* ablation of P-450 differed in male and female mice, all P-450 isoforms were affected regardless of gender.

To determine the pharmacological impact of the absence of *mdr1a* or *mdr1b* independently or simultaneously to P-450 mediated metabolism, we measured a catalytic activity that is a marker of CYP3A mediated metabolism, the 1′- and 4-hydroxylation of midazolam. Among the mice tested (all bred to an FVB background), the highest levels of midazolam hydroxylation activity were observed in *mdr1a/1b* (−/−) mice followed by *mdr1a* (−/−) (Fig. 5). Livers from *mdr1b* mice showed lower activity than observed in *mdr1a* (−/−) mice, but higher activity than in (+/+) mice (Fig. 5). Thus, in support of the immunoblot analysis of CYP3A (Fig. 4), *mdr1a* has the most dramatic impact on CYP3A mediated metabolism.

In addition, we examined mRNA expression of the major liver protein albumin (Fig. 6), a gene known to be inducible by glucocorticoids (Tonjes et al., 1992), and found that its expression was increased 1.8 ± 0.21-fold and 1.43 ± 0.30-fold in female and male *mdr1a/1b* (−/−) mice, respectively, compared with *mdr1a/1b* (+/+) mice (Fig. 6). This difference in female mice was statistically significant ($P < .05$). The phe-

TABLE 3

Mice housed in Amsterdam

Liver microsomes were analyzed by immunoblot with specific P450 antibodies and the bands were quantified by densitometry. The values from the (+/+) mice were assigned the number 1.0. Data are shown as mean ± S.D. of three to five animals per genotype per group with samples analyzed on multiple gels. Statistical difference between the (+/+) and (−/−) mice was assessed by a two-sided Student's *t* test with $P < .05$ as the limit of significance.

	mdr1a (FVB × 129/Ola) n = 3/genotype		*mdr1a* (FVB) n = 5/genotype		*mdr1b* (FVB) n = 5/genotype		*mdr1a/1b* (FVB) n = 5/genotype	
	(+/+)	(−/−)	(+/+)	(−/−)	(+/+)	(−/−)	(+/+)	(−/−)
Male								
CYP3A	1.0 ± 0.50	3.01 ± 0.48	1.0 ± 0.10	2.02 ± 0.23***	1.0 ± 0.10	1.47 ± 0.14***	1.0 ± 0.39	3.21 ± 0.49***
CYP1A	1.0 ± 0.88	2.54 ± 0.88	1.0 ± 0.17	1.72 ± 0.04***	1.0 ± 0.17	1.38 ± 0.33*	1.0 ± 0.2	3.96 ± 1.62***
CYP2B	1.0 ± 0.62	0.82 ± 0.65	1.0 ± 0.09	3.37 ± 0.86**	1.0 ± 0.09	2.53 ± 1.01*	1.0 ± 0.11	8.28 ± 1.62***
P450-R			1.0 ± 0.38	1.79 ± 0.16**	1.0 ± 0.38	2.16 ± 0.62	1.0 ± 0.7	2.72 ± 0.44**
CYP2D							1.0 ± 0.37	3.25 ± 0.37***
CYP4A							1.0 ± 0.85	4.19 ± 0.85***
Female								
CYP3A	1.0 ± 0.42	1.75 ± 0.25*					1.0 ± 0.42	4.50 ± 0.74***
CYP1A	1.0 ± 0.65	1.58 ± 0.16					1.0 ± 0.29	5.80 ± 2.23*
CYP2B	1.0 ± 1.27	3.26 ± 0.39					1.0 ± 0.02	11.5 ± 2.98**
P450-R							1.0 ± 0.07	2.56 ± 0.35**
CYP2D							1.0 ± 0.19	3.51 ± 1.17*
CYP4A							1.0 ± 0.8	2.30 ± 0.84

P-450-R, P450 reductase
* $P < .05$; ** $P < .01$; *** $P < .005$.

TABLE 4

Mice housed in the United States

Liver microsomes were analyzed by immunoblot with specific P450 antibodies and the bands quantified by densitometry. The values from the (+/+) mice were assigned the number 1.0. Data are shown as mean ± S.D. of three to five animals per genotype per group with samples analyzed on multiple gels. Statistical difference between the (+/+) and (−/−) mice was assessed by a two-sided Student's *t* test with $P < .05$ as the limit of significance.

	CF1 (untreated) n (+/+) = 5; (+/−) = 7; (−/−) = 10			*mdr1a* (FVB) n = 4/genotype		*mdr1a/1b* (FVB) n = 4/genotype	
	(+/+)	(+/−)	(−/−)	(+/+)	(−/−)	(+/+)	(−/−)
Male							
CYP3A	1.0 ± 0.71	1.66 ± 1.15	2.08 ± 1.18	1.0 ± 0.25	0.61 ± 0.25*	1.0 ± 0.18	0.79 ± 0.25
CYP1A				1.0 ± 0.07	0.92 ± 0.27		
CYP2B				1.0 ± 0.50	0.99 ± 0.51	1.0 ± 0.12	0.95 ± 0.10

	mdr1a (FVB) 12 wk n = 2/genotype		*mdr1a* (FVB) 17 wk n = 3/genotype		*mdr1a* (FVB) 23 wk n = 3/genotype	
	(+/+)	(−/−)[a]	(+/+)	(−/−)	(+/+)	(−/−)
Female						
CYP3A	1.0	1.23 ± 0.35	1.0 ± 0.42	2.11 ± 0.13*	1.0 ± 0.51	0.76 ± 0.37
CYP1A	1.0	1.08 ± 0.04	1.0 ± 0.15	1.00 ± 0.23	1.0 ± 0.23	0.72 ± 0.80
CYP2B	1.0	0.97 ± 0.51	1.0 ± 0.09	1.92 ± 0.21*	1.0 ± 0.14	1.00 ± 0.17

* $P < .05$; ** $P < .01$; *** $P < .005$.
[a], results are the mean ± the range.

notype described provides convincing evidence that *mdr1*-type P-glycoproteins can influence the hepatic expression of P-450s and, in fact, other liver genes.

***mdr1a* (−/−) and (+/+) Female Mice (FVB) Housed in the United States.** To further evaluate the effect of *mdr1a* on hepatic P-450 gene expression seen in the mice housed in Amsterdam, we analyzed additional groups of female *mdr1a* wild-type and homozygous mice obtained from a commercial vendor (Taconic Farms) and housed in the United States (at different times). These mice are genetically identical with those in Amsterdam, having originated from the same laboratory (Schinkel et al., 1994, 1997). Two of the groups (ages 12 and 23 weeks) housed in the United States displayed no difference in CYP3A or CYP2B expression between genotypes, whereas a third group of *mdr1a* (−/−) female mice (age 17 weeks) displayed a 2.11- and 1.92-fold higher expression of CYP3A and CYP2B, respectively, compared with *mdr1a* (+/+) mice (Fig. 7 and Table 4). CYP1A expression was unaffected by *mdr1a* genotype in 12- and 17-week-old female mice and was slightly decreased in 23-week-old (−/−) mice. Also noted was an age-dependent decrease in hepatic P-450 gene expression (Fig. 7). These results demonstrate that even among three different groups of genetically identical female *mdr1a* (−/−) mice housed in the United States environment, there can be different P-450 phenotypes. Importantly, multiple variables apply to the mice tested in this figure, including age, period in which the tissues were harvested, and genotypes. Thus, many factors may contribute to whether an effect of Pgp phenotype on P-450 is detected or not.

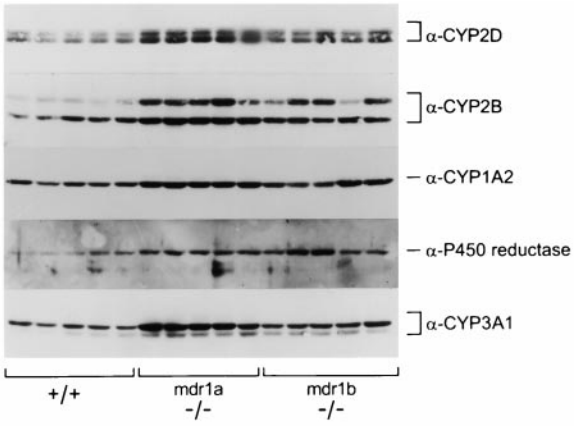

Fig. 3. Hepatic P-450s in male *mdr1a* and *mdr1b* (+/+) and (−/−) mice (FVB) housed in Amsterdam. Ten micrograms of microsomal protein from the livers of individual male *mdr1a* (+/+) or (−/−) or *mdr1b* (−/−) mice in a FVB background and 12 weeks of age were analyzed by immunoblot using antibodies against mouse CYP2D, rat CYP4A, CYP2B, CYP1A2, P-450 reductase, and CYP3A1.

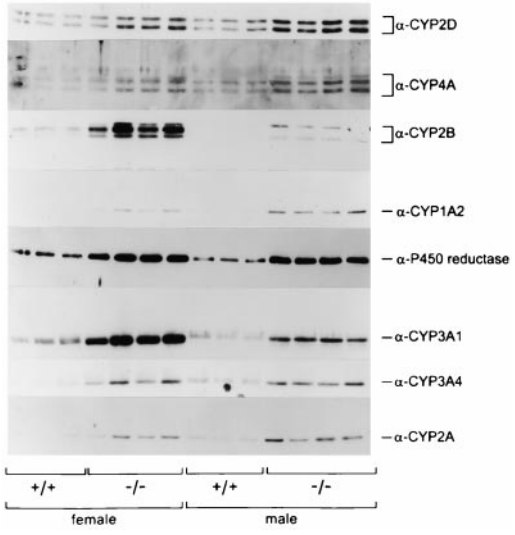

Fig. 4. Hepatic P-450s in male and female *mdr1a/1b* (+/+) and (−/−) mice housed in Amsterdam. Ten micrograms of microsomal protein from the livers of individual male and female *mdr1a/1b* (+/+) or (−/−) mice in a FVB background and 12 weeks of age were analyzed by immunoblot using antibodies against mouse CYP2D and CYP2A, rat CYP4A, CYP2B, CYP1A2, P-450 reductase, CYP3A1, and human CYP3A4.

***mdr1a* and *mdr1a/1b* (−/−) and (+/+) Male Mice (FVB) Housed in the United States.** Because female mice inherently have greater fluctuations in endocrine factors that could be influencing P-450 gene expression, we performed similar studies on male *mdr1a* (−/−), *mdr1a/1b* (−/−), and *mdr1* (+/+) mice all obtained from the commercial vendor and housed in the United States. To our surprise, P-450 protein levels were slightly higher in the *mdr1a* (+/+) than in the *mdr1a* (−/−) mice (Fig. 8A; Table 4). Consistent with

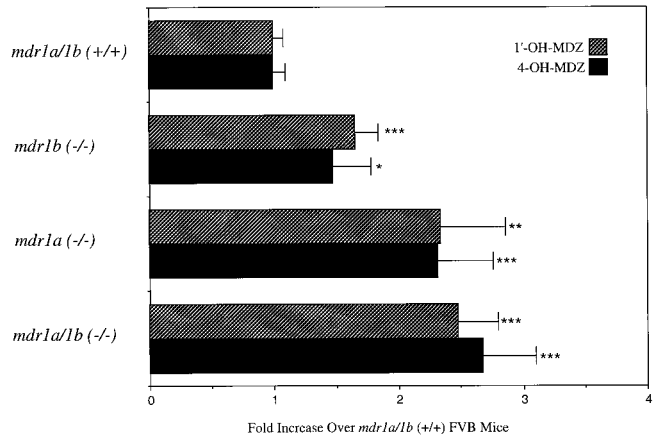

Fig. 5. CYP3A activity (midazolam 1′- and 4-hydroxylation) in liver microsomes of wild-type (+/+) and *mdr1a*, *mdr1b*, and *mdr1a/1b* (−/−) male mice housed in Amsterdam. Activities (nanomoles of product per milligram of microsomal protein per hour) from (+/+) mice were assigned the number 1.0. Results are the means ± S.D. of duplicate determinations of five mice per genotype. Statistical differences in midazolam hydroxylation in (+/+) animals were compared to knock-outs by a two-sided Student's *t* test with $P < .05$ as the limit of significance. *$P < .05$; **$P < .01$; ***$P < .005$.

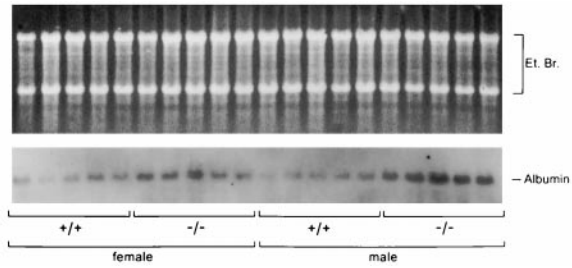

Fig. 6. Northern blot analysis of liver gene expression in *mdr1a/1b* (+/+) and (−/−) mice housed in Amsterdam. Ten micrograms of total RNA from livers of *mdr1a/1b* (+/+) and (−/−) male and female mice were resolved on a denaturing 1% agarose gel, stained with ethidium bromide, transferred to a nylon membrane, and hybridized with a rat albumin cDNA.

the lower amount of CYP3A immunoreactive protein in *mdr1a* (−/−) mice, the formation of 4-hydroxymidazolam and 1′-hydroxymidazolam were 0.70 ± 0.1-fold and 0.78 ± 0.1-fold lower in *mdr1a* (−/−) compared with (+/+) mice. Side-by-side comparison of CYP3A proteins in male mice of approximately the same age housed in Amsterdam or the United States (Fig. 8A) showed the consistent phenotype of an increase in CYP3A expression in *mdr1a* (−/−) mice housed in Amsterdam or a slight suppression of CYP3A in *mdr1a* (−/−) mice housed in the United States. The level of CYP3A and CYP2B were indistinguishable between the livers of *mdr1a/1b* (+/+) and (−/−) mice housed in the United States (Fig. 8B).

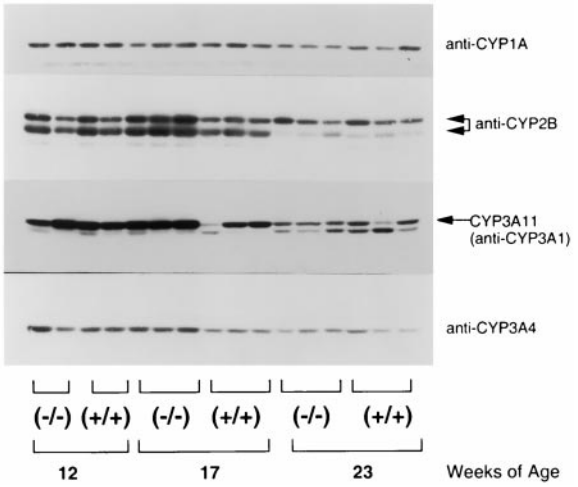

Fig. 7. Immunoblot analysis of P-450s in livers of female *mdr1a* (+/+) and (−/−) mice housed in the U.S. at different times. Ten micrograms of microsomal protein from the livers of individual female *mdr1a* (+/+) or (−/−) mice in a FVB background and 12, 17, and 23 weeks of age were analyzed by immunoblot using antibodies against rat CYP1A2, CYP2B, CYP3A1, and human CYP3A4.

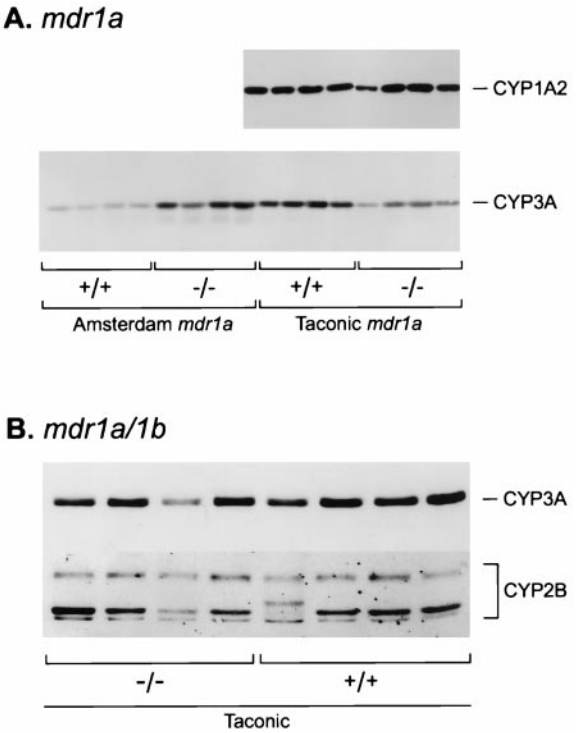

Fig. 8. Immunoblot analysis of P-450s in livers of 12-week-old male *mdr1a* and *mdr1a/1b* (+/+) and (−/−) mice in an FVB background housed in the U.S. A, ten micrograms of microsomal protein from the livers of individual *mdr1a* (+/+) and (−/−) mice housed in Amsterdam or from Taconic Labs and housed in the United States were analyzed by immunoblot using antibodies against rat CYP1A2 and CYP3A1. B, ten micrograms of microsomal protein from the livers of individual male *mdr1a/1b* (+/+) and (−/−) mice purchased from Taconic and housed in the United States were analyzed by immunoblot using antibody against rat CYP3A1 or anti-rat CYP2B.

Discussion

We tested the hypothesis that the membrane transport protein P-glycoprotein affects P-450 expression. To ascertain the functional importance of Pgp on P-450s, we specifically targeted protein expression as the relevant endpoint to measure. Results from *mdr1a* deficient mice from several different experimental models [1) *mdr1a* knockout mice in a 129/Ola × FVB outcross, as well as a pure FVB background, and 2) *mdr1a/1b* double knockout mice in a 99% pure FVB background, each housed in Amsterdam] confirm an important role for Pgp in the regulation of hepatic cytochrome CYP3A gene expression. Additionally, CF1 mice with a natural mutation in the *mdr1a* gene showed the same phenotype. These results also demonstrate that despite the fact that both *mdr1a* and *mdr1b* are expressed in liver, these proteins have distinguishable functional roles in influencing expression of P-450s, because it was primarily *mdr1a* that regulated hepatic P-450 expression, although *mdr1b* further affects expression of P-450s. The influence of Pgp was not limited to CYP3A; other P-450 families were also affected, particularly CYP2B in female mice. Taken together, these data support our hypothesis that Pgp has a major role in influencing hepatic P-450 expression, presumably by regulating the level of P-450 modulators in the liver.

Perhaps not surprisingly, the phenotypic consequences of *mdr1a* disruption varied depending on the environment in which the animals were housed, and also may have been subject to physiological changes in the mice. The results described in the male mice housed in Amsterdam differed from those of male *mdr1a* (−/−) and *mdr1a/1b* (−/−) mice housed in the United States. Although there are reports demonstrating that the phenotype seen in gene knockouts can vary with genetic background (Harvey et al., 1993), the *mdr1a* (−/−) and *mdr1a/1b* (−/−) FVB mice housed in the two different environments are genetically identical, having originated from the same laboratory. This is, to our knowledge, the first report showing such a dramatic effect of environment on phenotype in knockout mouse lines. Thus, it is important to routinely examine the P-450 phenotype in these mice because it could affect the outcome of pharmacological experiments that are increasingly performed using *mdr1* (−/−) mice.

Because *mdr1a* and *mdr1a/1b* (−/−) have no phenotype unless challenged with xenobiotics (Schinkel et al., 1994) and because mice lacking *mdr1* genes accumulate significantly higher concentrations of Pgp substrates [including glucocorticoids and xenochemicals (Schinkel et al., 1995)] in tissues normally expressing the *mdr1* transporters, the simplest explanation for our results is that *mdr1* deficient mice accumulate higher intrahepatic concentrations of ligands important

for regulation of P-450s. The most obvious consequence anticipated would be up-regulation of alternative mechanisms for steroid and drug clearance [i.e., induction of P-450s as seen in this study, and the compensatory increase in hepatic *mdr1b*, a gene also known to be regulated by steroids (Piekarz et al., 1993)] previously reported in *mdr1a* (−/−) mice (Schinkel et al., 1994). The elevation of P-450 in some groups/populations of *mdr1a* (−/−) mice demonstrates that in the absence of Pgp, the mice must accumulate some positive regulator in the liver. However, either an external signal varies (e.g., exposure to a certain dietary regulator) and/or an endogenous factor varies and may depend on the environment (e.g., the endocrine status of the mouse). Consequently, the phenotypic manifestations of *mdr1a* disruption on P-450 expression may be subject to both internal and external stimuli that do not remain static but are influenced by the environment of the animal.

Candidate *endogenous* molecules that could be regulating hepatic P-450 expression in *mdr1* knockout mice are numerous. Because so many classes of P-450s were affected, Pgp could be influencing a common P-450 endogenous regulator such as heme or steroids. Heme has been proposed to regulate P-450 mRNA and apoprotein levels (Dwarki et al., 1987). Although heme has not yet been tested as a Pgp substrate, bilirubin, the structurally similar heme breakdown product, is a putative substrate for *mdr1a* Pgp (Watchko et al., 1998). Steroids, particularly glucocorticoids, are well known regulators of the P-450s, including CYP3A (Schuetz and Guzelian, 1984) and CYP2B (Nemoto and Sakurai, 1995; Strom et al., 1996). Indeed, constitutive and glucocorticoid-inducible expression of mouse liver CYP2B requires the glucocorticoid receptor (Schuetz et al., in press) demonstrating an important role of these steroids and the glucocorticoid receptor in regulation of this cytochrome. Moreover, some of the P-450s [e.g., CYP1A (Mathis et al., 1989) and CYP3A (Hashimoto et al., 1993)] contain putative glucocorticoid receptor elements in their genes. Importantly, PXR, a transcription factor ligand activated by endogenous and exogenous steroids and drugs (e.g., rifampin) that transcriptionally activates the *CYP3A* genes has recently been identified. Ligand activators of PXR include endogenous steroids such as cortisol, corticosterone, pregnenolone, and estradiol (Blumberg et al., 1998). Because PXR ligands up-regulate many P-450s, phase II enzymes, and drug efflux transporters, and because consensus PXR binding motifs have been identified in the 5′-flanking sequences of some of these orthologous genes in other species (Blumberg et al., 1998), it is likely that Pgp affects the cellular bioavailability of PXR ligands in mice. In keeping with this model, Pgp is known to transport a number of these same effector molecules important for PXR and glucocorticoid receptor activation, including endogenous steroids [cortisol, corticosterone, aldosterone, the androgen precursor dehydroepiandrosterone, pregnenolone, and 17-hydroxyprogesterone (Schinkel et al., 1995; Barnes et al., 1996)]. Moreover, we have previously shown that stress levels of endogenous glucocorticoids [e.g., 3–9 $\times$ 10^{-7} M corticosterone in male and female rats (Schuetz et al., 1992)] are sufficient to induce CYP3A in primary rat hepatocytes (Schuetz and Guzelian, 1984), and it is possible such levels of glucocorticoids are realized in the livers of *mdr1* null mice. Indeed, the increase in albumin mRNA expression in the *mdr1a/1b* (−/−), a gene known to be induced by glucocorticoids (Tonjes et al., 1992), supports the model that in the absence of Pgp there is an increase in the hepatic expression of these steroids. These candidate effector molecules could either directly or indirectly affect P-450 expression via effects on, for example, a common heme pool or a common hepatic transcription factor. On the other hand, the results may reflect Pgp transport of multiple effector molecules that independently regulate individual P-450 isoforms. The consequences of ovariectomy or adrenalectomy of *mdr1a/1b* (−/−) mice to remove some endogenous steroids may shed some light on the contribution of these hormones to the P-450 phenotype in these mice.

The list of candidate *exogenous* stimuli that are Pgp-transported and that could influence P-450s is extensive and includes regulators of dietary origin. Pesticides are known inducers of P-450 gene expression (Schuetz et al., 1986), and have also been documented to interact with Pgp (Schinkel et al., 1994). Both laboratory chow and wood-chip bedding can also affect expression of some hepatic P-450s (Schmidt et al., 1996). Given the identification of estradiol and phytoestrogens as potent and efficacious stimulators of PXR (Blumberg et al., 1998), it is relevant that several reports have found high (and variable) concentrations of estrogenic components in animal feed (reviewed in Boettger-Tong et al., 1998). Indeed, animal feeds routinely are abundant in soy and alfalfa, the richest natural sources of coumestans (Boettger-Tong et al., 1998), which is particularly germane because coumestrol, a dietary phytoestrogen, is the most efficacious activator of PXR identified (Blumberg et al., 1998). Estradiol (Barnes et al., 1996), and perhaps phytoestrogens, interact with Pgp. Unfortunately, the concentrations of phytoestrogens in animal chow are not routinely measured. However, the variations in dietary (and physiological) concentrations of P-450 regulators may help to explain the fact that even among the male and female mice housed in the United States, we observed different P-450 phenotypes between some groups of *mdr1a* (+/+) and (−/−) mice. Indeed, two other recent reports using mice with *mdr1a* ablation housed in the United States have failed to find differences in 'CYP3A' protein expression (Kwei et al., 1999; Perloff et al., 1999). Intriguingly, however, one of these reports (Perloff et al., 1999) found elevated formation of α-hydroxy-midazolam and triazolam and several metabolites of dexamethasone in liver microsomes of *mdr1a* (−/−) compared with (+/+) mice. This finding is indicative of an up-regulation of some P-450 in the livers of the mice lacking Pgp, similar to our results of P-450 induction in the livers of *mdr1a* and *mdr1a/1b* (−/−) mice housed in Amsterdam. In total, these findings support the important role of *mdr1* in modulating the intracellular disposition of P-450 effector molecules. Further studies will be required to determine the exact dietary, physiological, and environmental Pgp substrates regulating P-450s in this model.

In some male *mdr1a* (−/−) mice housed in the United States, there was decreased CYP3A expression. The down-regulation of CYP3A implies that the hepatic concentration of some negative regulator of CYP3A is elevated in some of these mice. This phenotype would be consistent with P-450 suppression by cytokines (Abdel-Razzak et al., 1993; Thal et al., 1994), some of which are putative substrates for *mdr1* (Drach et al., 1996).

What do these results mean to the utility of using *mdr1a* or

mdr1a/1b knockout mice for drug disposition studies? The enhanced concentration of hepatic P-450s may explain, in part, the modest influence of hepatic *mdr1a* (compared with intestinal or brain) to drug disposition (Schinkel et al., 1994, 1995, 1997) observed in the knockout mice and suggests that the importance of hepatic *mdr1a* to drug disposition may be underestimated because of the compensatory increases in *mdr1b* and P-450 in this tissue. Nevertheless, numerous reports analyzing the disposition of some drugs in *mdr1a* (−/−) mice demonstrates a unique role for *mdr1a* in the intestine and brain that clearly cannot be compensated for by up-regulation of hepatic P-450s.

In summary, the phenotype described provides convincing evidence that there are checks and balances among various components of endogenous steroid and xenochemical elimination and positions *mdr1*-type Pgp as an important upstream regulator of hepatic P-450 expression. The concept that Pgp can influence P-450 expression expands our understanding of the cooperative interrelationship between two of the most important factors in drug and steroid elimination. Finally, although these studies were performed in mice, human MDR1 (and human PXR) have similar steroid substrate (and ligand) specificities. Moreover, we and others have demonstrated that there is substantial interindividual variation in expression of human MDR1 (Schuetz et al., 1995; Lown et al., 1997). Therefore, we propose that individual differences in expression of MDR1/Pgp in humans are likely to regulate ligand availability for PXR, the glucocorticoid receptor, and other signaling pathways, and thus could be an important regulator of human P-450 expression. These results also have implications for patients receiving drugs that inhibit or induce Pgp, because this could additionally affect P-450 expression and thus the disposition of drugs.

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Erratum

In an article by Schuetz et al. (2000), Tables 3 and 4 printed with the data in the body of the tables not aligned correctly [Schuetz EG, Umbenhauer DR, Yasuda K, Brimer C, Nguyen L, Relling MV, Schuetz JD and Schinkel AH (2000) Altered expression of hepatic cytochromes P-450 in mice deficient in one or more *mdr1* genes. *Mol Pharmacol* **57:**188–197]. The corrected tables follow. The HTML and PDF versions of the online journal are correct. We regret any inconvenience caused by this error.

TABLE 3

Mice housed in Amsterdam

Liver microsomes were analyzed by immunoblot with specific P450 antibodies and the bands were quantified by densitometry. The values from the (+/+) mice were assigned the number 1.0. Data are shown as mean ± S.D. of three to five animals per genotype per group with samples analyzed on multiple gels. Statistical difference between the (+/+) and (−/−) mice was assessed by a two-sided Student's *t* test with $P < .05$ as the limit of significance.

	mdr1a (FVB × 129/Ola) n = 3/genotype		*mdr1a* (FVB) n = 5/genotype		*mdr1b* (FVB) n = 5/genotype		*mdr1a/1b* (FVB) n = 5/genotype	
	(+/+)	(−/−)	(+/+)	(−/−)	(+/+)	(−/−)	(+/+)	(−/−)
Male								
CYP3A	1.0 ± 0.50	3.01 ± 0.48	1.0 ± 0.10	2.02 ± 0.23***	1.0 ± 0.10	1.47 ± 0.14***	1.0 ± 0.39	3.21 ± 0.49***
CYP1A	1.0 ± 0.88	2.54 ± 0.88	1.0 ± 0.17	1.72 ± 0.04***	1.0 ± 0.17	1.38 ± 0.33*	1.0 ± 0.2	3.96 ± 1.62***
CYP2B	1.0 ± 0.62	0.82 ± 0.65	1.0 ± 0.09	3.37 ± 0.86**	1.0 ± 0.09	2.53 ± 1.01*	1.0 ± 0.11	8.28 ± 1.62***
P450-R			1.0 ± 0.38	1.79 ± 0.16**	1.0 ± 0.38	2.16 ± 0.62	1.0 ± 0.7	2.72 ± 0.44**
CYP2D							1.0 ± 0.37	3.25 ± 0.37***
CYP4A							1.0 ± 0.85	4.19 ± 0.85***
Female								
CYP3A	1.0 ± 0.42	1.75 ± 0.25*					1.0 ± 0.42	4.50 ± 0.74***
CYP1A	1.0 ± 0.65	1.58 ± 0.16					1.0 ± 0.29	5.80 ± 2.23*
CYP2B	1.0 ± 1.27	3.26 ± 0.39					1.0 ± 0.02	11.5 ± 2.98**
P450-R							1.0 ± 0.07	2.56 ± 0.35**
CYP2D							1.0 ± 0.19	3.51 ± 1.17*
CYP4A							1.0 ± 0.8	2.30 ± 0.84

P-450-R, P450 reductase

* $P < .05$; ** $P < .01$; *** $P < .005$.

TABLE 4

Mice housed in the United States

Liver microsomes were analyzed by immunoblot with specific P450 antibodies and the bands quantified by densitometry. The values from the (+/+) mice were assigned the number 1.0. Data are shown as mean ± S.D. of three to five animals per genotype per group with samples analyzed on multiple gels. Statistical difference between the (+/+) and (−/−) mice was assessed by a two-sided Student's *t* test with $P < .05$ as the limit of significance.

	CF1 (untreated) n (+/+) = 5; (+/−) = 7; (−/−) = 10			*mdr1a* (FVB) n = 4/genotype		*mdr1a/1b* (FVB) n = 4/genotype	
	(+/+)	(+/−)	(−/−)	(+/+)	(−/−)	(+/+)	(−/−)
Male							
CYP3A	1.0 ± 0.71	1.66 ± 1.15	2.08 ± 1.18	1.0 ± 0.25	0.61 ± 0.25*	1.0 ± 0.18	0.79 ± 0.25
CYP1A				1.0 ± 0.07	0.92 ± 0.27		
CYP2B				1.0 ± 0.50	0.99 ± 0.51	1.0 ± 0.12	0.95 ± 0.10

	mdr1a (FVB) 12 wk n = 2/genotype		*mdr1a* (FVB) 17 wk n = 3/genotype		*mdr1a* (FVB) 23 wk n = 3/genotype	
	(+/+)	(−/−)[a]	(+/+)	(−/−)	(+/+)	(−/−)
Female						
CYP3A	1.0	1.23 ± 0.35	1.0 ± 0.42	2.11 ± 0.13*	1.0 ± 0.51	0.76 ± 0.37
CYP1A	1.0	1.08 ± 0.04	1.0 ± 0.15	1.00 ± 0.23	1.0 ± 0.23	0.72 ± 0.80
CYP2B	1.0	0.97 ± 0.51	1.0 ± 0.09	1.92 ± 0.21*	1.0 ± 0.14	1.00 ± 0.17

* $P < .05$; ** $P < .01$; *** $P < .005$.

[a], results are the mean ± the range.