0031-3998/05/5806-1284
PEDIATRIC RESEARCH
Vol. 58, No. 6, 2005
Printed in U.S.A.

Ontogeny of Renal P-glycoprotein Expression in Mice: Correlation with Digoxin Renal Clearance

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ABSTRACT

Digoxin is eliminated mainly by the kidney through glomerular filtration and P-glycoprotein (P-gp) mediated tubular secretion. Toddlers and young children require higher doses of digoxin per kilogram of bodyweight than adults, although the reasons for this have not been elucidated. We hypothesized there is an age-dependant increase in P-gp expression in young children. The objectives of this study were to elucidate age-dependant expression of renal P-gp and its correlation with changes in the clearance rate of digoxin. FVB mice were killed at different ages to prepare total RNA for P-gp expression studies. Semi-quantitative RT-PCR was conducted to analyze mdr1a and mdr1b ontogeny in the kidney at: birth, 7, 14, 21, 28 and 45-d old adults. The pharmacokinetics of digoxin (7 μg/kg) was studied in mice of the same age groups. Newborn and Day 7 levels of both mdr1a and mdr1b were marginal. Day 21 mdr1b levels were significantly higher than both Day 14 and Day 28 levels. Digoxin clearance rates were the highest at Day 21, with significant correlation between P-gp expression and clearance values. Increases in digoxin clearance rates after weaning may be attributed, at least in part, to similar increases in P-gp expression. **(*Pediatr Res* 58: 1284–1289, 2005)**

Abbreviations

IDV, integrated density value
p-gp, P-glycoprotein,

The kidney is a major port of exit from the body for numerous drugs and/or their metabolites. The ontogeny of renal handling of drugs has been considered in depth only in the context of low clearance rates in newborn infants in both clinical and experimental studies. Yet, there is evidence that in the post neonatal period, the young child needs much larger doses of renally excreted drugs compared with older children and adults. In the case of digoxin, which is frequently used in pediatrics and excreted mostly by the kidney, a neonate typically requires a daily dose of 5 μg/kg, the toddler and young child need 10–12 μg/kg per day, whereas older children and adults need 8–10 μg/kg and 3–5 μg/kg respectively (1).

The mechanisms underlying these developmental changes have not been elucidated. The kidney clears most of the body load of digoxin by both glomerular filtration and tubular secretion, with digoxin half-lives in children varying consistently with renal clearance (2,3). Tubular secretion of digoxin is mediated by the P-glycoprotein (P-gp) transporter, encoded by the mdr1 gene (4). Presently, the ontogeny of renal P-gp has been sparsely studied and never linked directly to the elimination characteristics of digoxin, or other P-gp substrates. Whereas P-gp in humans is encoded by the one gene, *MDR1*, there are two genes responsible for encoding this drug transporter in murine kidney: mdr1a and mdr1b (5,6). The two genes are believed to have risen through gene duplication from a common precursor (7). Mdr1b was shown to be expressed at high levels in the pregnant uterus and the adrenal gland and expressed at intermediate levels in kidney and heart tissue (5). Longer exposures also revealed limited expression in muscle, brain, liver, spleen, and lungs. On the other hand, mdr1a is expressed at high levels in the intestine and at intermediate levels in the brain, heart, and lung and at low levels in muscle and kidney, with some liver and spleen levels (5). The two genes appear to work in concert in mice, with mdr1b expression being up-regulated in mdr1a(-/-) knockout mice (8). The murine equivalent of the phospholipid transporter *MDR3* is mdr2, which is predominantly expressed in liver, muscle, and spleen (9).

Schiengold *et al.* studied the development of mdr1, mdr2 and mdr3 in both BALB/c and C57BL/6 mice using RT-PCR (alternative nomenclature uses mdr1 for mdr1b and mdr3 for mdr1a) (10). The kidney as an organ was characterized by the

Received January 31, 2005; accepted May 31, 2005.
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Supported by a grant from the Canadian Institutes of Health Research (CIHR). Dr. Gideon Koren is holder of the Ivey Chair in Molecular Toxicology, University of Western Ontario.

DOI: 10.1203/01.pdr.0000188697.99079.27

presence of mdr3 and mdr1 in high frequency in all age groups, when compared with other organs. Although this study reveals the distribution of all three mdr isoforms in murine renal ontogeny, due to lack of a quantitative PCR method, the authors could not conclude anything about the relative amounts of expression during development.

The primary objective of the present study was to characterize the ontogeny of renal P-gp and of digoxin clearance in mice, and the correlation between the two. During growth and development, organ sizes can differ in relationship to body size. If the kidney in a particular stage of development is larger in proportion to the animal's body size, this could be an important determinant to explain any functional differences among age groups. Hence our second objective was to investigate whether the ratio of kidney weight to body weight varies across the age groups in question.

METHODS

Animals. We chose FVB mice because P-gp-mediated tubular secretion of digoxin has been documented in this strain, (11,12) exhibiting P-gp expression at birth (13). This protocol was approved by the Animal Care Committee at the Hospital for Sick Children. Male and female FVB mice of mating age were purchased from Charles River Laboratories (Wilmington, Massachusetts). Mice were housed in static microisolators in a conventional mouse room at the Laboratory Animal Services Facility at the Hospital for Sick Children. The animals were maintained on standard laboratory chow (Purina Rodent Chow 1000, Purina Mills, St. Louis, Missouri) and were allowed food and water *ad libitum*. The mice were observed daily to check food, water and health status. The facility was on a 12-h dark/ 12-h light cycle. Mice were weaned at 21 d of age.

The renal expression of P-gp was assessed in offspring (4 male, 4 female) at the following postnatal ages: birth, 7 d, 14 d, 21 d, 28 d and 45 d-old adults. The age groups chosen represent the neonatal, pups, weaning and young adult stages of development, a full spectrum which will allow appropriate characterization of the ontogeny of drug transport. For ages 14 d old and up, kidneys from one mouse were used per PCR run. For Day 7, kidneys from two mice were pooled and at birth, kidneys from four mice were pooled.

Total RNA extraction. Kidneys were dissected from mice at each age group, were weighed and immediately placed in RNA Later (Ambion Inc., Austin, Texas) on ice. Tissue was minced on ice, homogenized with a plastic eppendorf-tube homogenizer immediately in 1 mL of TRIzol® (Invitrogen, Carlsbad, California) per 100 mg of tissue. RNA was extracted according to the TRIzol® protocol. The quantity and quality of the RNA extraction was determined by measuring absorbance at 260 nm and 280 nm in a Beckman DU® 530 spectrophotometer (Beckman Coulter Canada Inc., Mississauga, Ontario). 1 μg of each RNA sample was run on a 1% agarose gel containing 10 μL ethidium bromide in a 0.5× TBE buffer for 1 h at 100 V to check the integrity of the extracted RNA. The gels showed the RNA to be intact, with only two bands appearing: the 28S and 18S ribosomal subunits.

Reverse transcriptase–polymerase chain reaction (RT-PCR). Reverse transcriptase-polymerase chain reaction is a highly sensitive technique for amplifying the initial amount of mRNA, which can then be used for quantitation. In semi-quantitative RT-PCR experiments, the PCR results must be normalized to an internal standard transcript, which is amplified in parallel. The one chosen for this study is β-actin.

cDNA synthesis. cDNA was synthesized using 2 μg of a 1 μg/μL total RNA stock, 1 μL of random primer (Amersham Pharmacia Biotech, Piscataway, New Jersey), and 17 μL of DEPC-treated double-distilled water for a total volume of 20 μL. This was incubated at 65°C for 10 min and then kept on ice for 2 min. 8 μL of 5 × reaction buffer (50 mM Tris-HC1 pH 8.3, 75 mM KC1, 3 mM $MgC1_2$ in a final volume of 40 μL) was added (Invitrogen, Carlsbad, California), 60 units of the RNAse inhibitor RNAguard™ (Amersham Pharmacia Biotech, Piscataway, New Jersey), 10 mM DTT (Invitrogen, Carlsbad, California), 0.5 mM dNTP (Invitrogen, Carlsbad, California), 2 μL DEPC-treated double-distilled water and 0.4 units Superscript II Rnase H reverse transcriptase (Invitrogen, Carlsbad, California) in a final volume of 20 μL. The reaction was incubated for 1 h at 37°C, then for 10 min at 65°C, and lastly on ice for 2 min. cDNA was then stored at -80°C until further processed.

Primers. For the PCR reaction, primers specific for mdrla (gi 199104) M33581 and mdrlb (gi199100) M14757 were designed. Since mdrla has 92% overall homology with mdrlb, (6) BLAST 2 sequence was used to align the two sequences to find the areas that had the least homology. For mdrla, mdrlb and the housekeeping gene β-actin, the primers were chosen so that they span one or more introns within the gene. The primers were commercially synthesized at the DNA Synthesis Centre, Hospital for Sick Children. Restriction analysis was conducted to verify PCR product extracted from an agarose gel and cloned into the pGEM®-T-Easy vector (Promega, Madison, Wisconsin). Restriction analysis was performed using *Pvu*II (mdrlb) or *Eco*RI (mdrla), which cleaved the PCR fragments only once as expected. Alternatively, the extracts were also sequenced at the DNA Sequencing Facility (Centre for Applied Genomics, Hospital for Sick Children) and then analyzed using BLAST.

The primer sequences designed were: mdrlb: sense 5′ GGATCCCAGAGTGACACTGAT 3′, anti-sense 5′ GATCTCAAGCTGTTTCTTGTCC 3′, expected product size 747bp; mdrla: sense 5′ GATCAACTCGCAAAAGCATCTG 3′, anti-sense 5′ CCACTCCAGCTATCGCAATG 3′, expected product size 624bp; β-actin: sense 5′ CTACAATGAGCTCCGTGTGG 3′, anti-sense 5′ TAGCTCTTCTCCAGAGAGGA 3′, expected product size 450bp.

Calibration experiments. To accurately study changes in P-gp RNA expression due to ontogeny, three parameters were calibrated: the amount of RNA used for cDNA synthesis, the volume of cDNA used for RT-PCR, and the PCR amplification cycle length. Mdr1a was amplified to 29 cycles, mdrlb was amplified to 28 cycles, and β-actin to 31 cycles.

PCR reaction. The PCR reaction mixture contained Expand High Fidelity buffer without $MgCl_2$ (Roche Diagnostics, Mannheim, Germany), 0.2 mM dNTP (Invitrogen, Carlsbad, California), 2.5 mM $MgCl_2$ (Invitrogen, Carlsbad, California), 100 ng of each primer and 3.5 units of Expand High Fidelity enzyme (*Taq* and Tgo DNA polymerases) (Roche Diagnostics, Mannheim, Germany). 3 μL of cDNA was added last to make a final reaction volume of 50 μL. PCR reactions for each gene and β-actin were conducted simultaneously for all age groups.

The reactions were run in a Applied Biosystems GeneAmp PCR System 2400 thermocycler (Applied Biosystems, Foster City, California). The PCR program consisted of 4 min at 94°C followed by denaturation at 94°C for 30 s, annealing at 60°C for 30 s and elongation at 72°C for 1 min. 40 μL of the PCR products from the different age groups and the β-actin standard were then separated on the same 2% agarose gel containing ethidium bromide in 0.5× TBE buffer. The gel was run for 1.5 h at 100 V against 10 μL of a 100 bp DNA Ladder (Invitrogen, Carlsbad, California).

Gel analysis. The agarose gels were read in a UVP White/UV Transilluminator (UVP Inc., Upland, California) and scanned using Scion Image 1.59 (Scion Corporation, Frederick, Maryland). The ethidium-stained bands of the PCR products were analyzed using AlphaEase version 5.5 (Alpha Innotech, San Leandro, California) at the Imaging Center at the Hospital for Sick Children. The program calculated the Integrated Density Value (IDV) of the stained bands relative to a background band of the same size chosen from an area of the gel with no staining.

Correction for kidney weight and body weight. To investigate if kidney weight *versus* body weights varied across the age groups in question, the kidneys and bodies of 3 male and 3 female FVB mice of each age group (except newborn) were weighed on the same balance. Three newborns were weighed as well. The ratio of mean gram kidney weight to mean gram bodyweight was calculated. The IDV values obtained from the PCR experiments were then corrected by their respective kidney to bodyweight ratios.

Pharmacokinetic studies. Pharmacokinetics studies of digoxin were carried out in FVB mice of the same age groups as the P-gp expression studies. Mice were administered a bolus dose of digoxin (Lanoxin, GlaxoSmithKline, Research Triangle Park, North Carolina) intraperitoneally (7 μg/kg). Doses at even higher than these levels have been shown to result in nontoxic serum concentrations (11). Digoxin levels in serum were determined by the Technicon Immuno 1® assay (Bayer Corporation, Tarrytown, New York) at the Hospital for Sick Children Core Laboratory in Toronto at 7 time points: 0.25h, 0.5h, 1h, 2h, 3h, 4h and 5h. At each time point, 6 mice of each age group were killed under anesthesia by lethal bleed. The samples were kept on ice and allowed to clot. They were then centrifuged at 2000 rpm for 10 min to separate out the plasma and subsequently analyzed.

We characterized the area under the digoxin concentration-time curve (AUC). Standard noncompartmental methods were used to calculate pharmacokinetic data using WinNonlin 4.01 (Pharsight Corporation, Mountain View, California). The terminal half-life of digoxin was calculated from the elimination phase. Clearance rate was calculated as the ratio between the administered dose and the AUC from zero to infinity, extrapolated from the elimination phase.

Statistical analysis. Comparison of P-gp expression and clearance rates across age groups was conducted using SigmaStat® version 2.03 (SPSS Inc., Chicago, Illinois). Descriptive statistics were performed on all samples. One-way Kruskal Wallis analysis of variance was conducted. When statistical

significance was reached among groups ($p < 0.05$), a Tukey's posthoc test was performed to determine specific age group differences.

RESULTS

Mdrlb. The mdrlb results were first normalized to the housekeeping gene β-actin and then plotted against the age group. A representative PCR gel and chart is shown in Fig. 1 and Fig. 2 ($n = 8$). The RNA expression pattern of mdrlb as determined by PCR was minimal at birth, about 11% of adult levels. On Day 7 it rose up slightly in expression (19% of adult expression), but was not significantly different from that at birth. Between Day 7 and Day 14, mdrlb levels rose dramatically to 85% of adult levels. In fact, from Day 14 onwards, all age groups had increases in expressions that were significantly different from birth and the first week of life (Fig. 2).

Mdrlb RNA levels on Day 14 continue to increase until they peak at Day 21, at 132% of the expression found in adult kidneys. However, from Day 21 to Day 28, levels decrease down to as low as they were on Day 14 (62% of adult levels). This dip is temporary, as further growth and development see RNA expression increasing again from Day 28 to adult levels, but not achieving significance. The peak seen at Day 21 is both statistically higher than levels at Day 14 ($p = 0.01$) and at Day 28 ($p = 0.015$). Even with the escalation from Day 28 once the mice reach adulthood, adult mdrlb RNA levels are lower than they were on Day 21. Neither the RNA expression levels at Day 28 nor adulthood was statistically different from those at Day 14 (Fig. 2).

Mdrla. The representative PCR gel and chart depicting mdrla IDV values normalized to β-actin, and compared by age group is also shown in Fig. 1 and Fig. 2 ($n = 8$). Unlike the results from mdrlb, the newborn levels of mdrla were significantly different from those found on Day 7 ($p = 0.01$). But similar to mdrlb, the RNA levels at birth and Day 7 were significantly lower than all ages Day 14 and up ($p < 0.02$), at 19% and 31% of adult levels respectively.

From relatively minimal levels in the first week of life, mdrla displayed a steadier rise in expression levels than mdrlb.

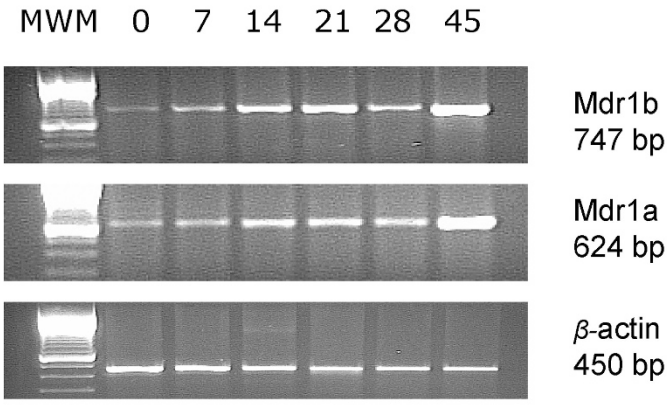

Figure 1. A representative PCR gel of mdr1b, mdr1a and β-actin expression. Total RNA was prepared from FVB kidneys at different ages: birth, 7 d, 14 d, 21 d, 28 d and 45-d old adults. Semi-quantitative RT-PCR was conducted to analyze mdr1a and mdr1b ontogeny ($n = 8$). PCR products from the different age groups and the housekeeping gene, β-actin, were then separated on the same agarose gel.

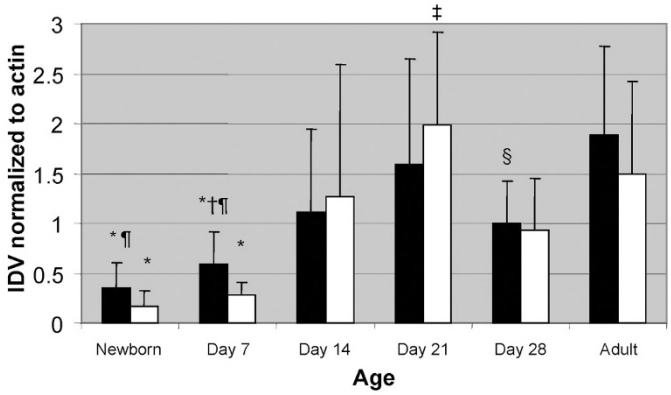

Figure 2. Expression of mdr1b and mdr1a normalized to β-actin, as a function of age (mean ± SD, $n = 8$). The mdrlb (□) and mdr1a (■) IDV results were first normalized to the housekeeping gene β-actin, and then plotted against the age group. One-way Kruskal Wallis analysis of variance was conducted. Tukey's posthoc analysis was undertaken to determine specific age group differences when statistical significance was reached among groups ($p < 0.05$). * Newborn and Day 7 mdr1a and mdr1b levels were significantly less than all other age groups ($p < 0.005$) † Day 7 mdr1a levels were significantly higher than newborn ($p = 0.01$). ‡ Day 21 mdr1b levels were significantly higher than Day 14 ($p = 0.01$) and Day 28 ($p = 0.015$), but not adult. § Day 28 mdr1a levels were significantly different from adult ($p < 0.03$) ¶ Overall, levels of mdrla was significantly higher than mdrlb levels only in newly-born mice and mice at Day 7 ($p < 0.05$).

Levels on Day 14 (60% of adult) rose progressively to Day 21 (85% of adult), but were followed with a drop to levels at Day 28 (53% of adult). Mice at 28 d had mdrla levels that were significantly different from adult levels ($p = 0.02$). Expression at Day 14 was not significantly different from Day 21, Day 28 or Adult. The same higher mdrla RNA expression at Day 21 relative to Day 14 and Day 28 was observed. This was as seen in mdrlb; however mdrla levels at Day 21 did not reach significance from either Day 28 or adult.

Mdrla versus Mdrlb. The ontogeny of mdrla was significantly different from mdrlb only in newly-born mice and mice at Day 7 ($p < 0.05$) (Fig. 2). Levels of mdrla were approximately 2-fold greater than its gene counterpart at the start of life. At all other age groups investigated, mdrla and mdrlb had similar ontogenic RNA expression patterns.

Kidney weight versus bodyweight ratio. Kidney weight was linearly correlated to body weight at each stage of ontogeny, and highly similar between male and female mice (Fig. 3). To correct for any effect these changes may have on our PCR results, the ratio of kidney weight to mean gram bodyweight was calculated. Figure 4 presents correlations between the calculated kidney to body weight ratio and the expression of mdrlb and mdrla. The IDV values obtained from the PCR experiments were then corrected by their respective kidney to bodyweight ratios. We found that there was no difference between those PCR results obtained before the body weight correction and those obtained after.

Digoxin pharmacokinetics. Table 1 details the pharmacokinetic values of digoxin clearance. At birth, pups had very little to no ability to excrete digoxin. Digoxin half-life was long, the AUC was very large, and the clearance was minimal. Clearance rates increased by Day 14. There was a noteworthy enhancement in clearance by Day 21. At the time of weaning,

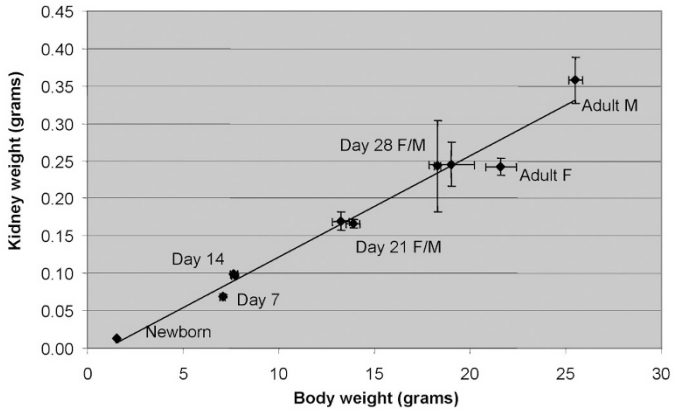

Figure 3. Graphical representation of the ratio of kidney weight in grams plotted against body weight in grams (mean ± SD, $n = 3$). Kidneys and bodies of 3 male and 3 female FVB mice of each age group (except newborn) were weighed on the same balance. Three newborns were weighed as well. The ratio of mean gram kidney weight to mean gram bodyweight was calculated to determine whether kidney weight varied *vs* body weight during postnatal development. A constant ratio of kidney weight to body weight was found ($r^2 = 0.974$).

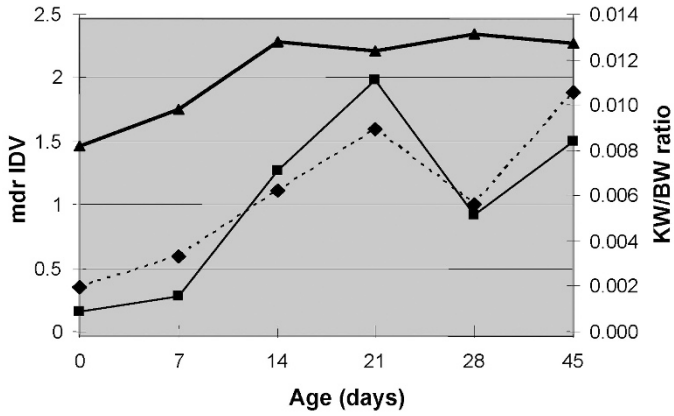

Figure 4. Correlation between age, mdr1b and mdr1a expression and the kidney to body weight ratio. Graphical representation of the changes in mdr1b (■) and mdr1a (◆)expression and that of the calculated kidney to body weight ratio (▲), as a function of age. Mdr1b and mdr1a displayed an ontogeny of expression, while the ratio of kidney weight to body weight remained relatively constant.

mice exhibited a greater clearance and elimination rate constant, and a significantly lower AUC.

In contrast, by Day 28, clearance rate slowed down to lower than that of Day 21. When we plotted our digoxin pharmacokinetic data against our mdr expression data, we found a statistically significant correlation between expression of mdr1b and mdr1a and the clearance rate of digoxin (Fig. 5).

Table 1: *Pharmacokinetic Parameters of Digoxin clearance in mice (n = 6)*

Age	Elimination Constant Ke	AUC (hour.μg/L)	Clearance (L/kg.hour)
Newborn	0.0119	302.1	0.00003
Day 7	0.2110	12.9	0.0034
Day 14	0.2230	23.4	2.986
Day 21	0.3976	9.8	7.150
Day 28	0.2327	13.1	5.037
Adult	0.3212	9.6	7.273

DISCUSSION

In this study, two hypothetical frameworks were examined to explain why toddlers have the highest clearance rates of digoxin, necessitating doubling of the dose compared with adults. Our primary hypothesis was that this is due in part to an increase in renal P-gp expression. In parallel, we wished to examine the possibility that at this age the kidney is proportionally larger in proportion to total body weight.

The role of renal P-glycoprotein in the tubular secretion of digoxin has been extensively documented *in vitro* and *in vivo*, in both animals and humans (14–20). In experiments in mouse proximal tubules perfused *in vitro*, Tsuruoka and colleagues have shown that the addition of verapamil, a P-gp inhibitor, resulted in substantial decrease in digoxin tubular secretion (14). This effect was not exhibited in mdr1a/1b(-)(-) mice. *In vitro* studies also showed that quinidine, a P-gp inhibitor, decreased the clearance of digoxin in wild type mice but not in mdr1a(-/-) mice (15).

When we normalized our mdr1b we found minimal RNA expression at birth, which rose slightly at Day 7. At 21 d of age, levels had exceeded adult expression. This indicates postnatal regulation of P-gp. Croop *et al.*, in their characterization of the tissue-specific localization of mdr genes, reported that mdr1b had higher expression in the kidney than mdr1a (5). Our mdr1a results exhibited a very similar trend, although mdr1a displayed a steadier rise in expression levels than mdr1b. Mdr1a levels at Day 21 were higher than Day 14 or 28. Here however, levels at Day 21 were not different from adult mice.

Other groups have investigated the renal ontogeny of P-gp to some extent. Dutt *et al.*, found a steady increase of mdr1a and mdr1b (normalized to β-actin) in murine kidney, from levels that were virtually undetectable at birth (21). The group detected abruptly high levels of mdr1b at 21 d (21), but no formal statistical analysis was presented. Rosati *et al.* also demonstrated regulation of P-gp postnatally (22). They found that levels of both mdr1a and mdr1b at birth had significant increases from postnatal Day 6 onwards in the kidney, which corresponds to our Day 7. However, they found that none of

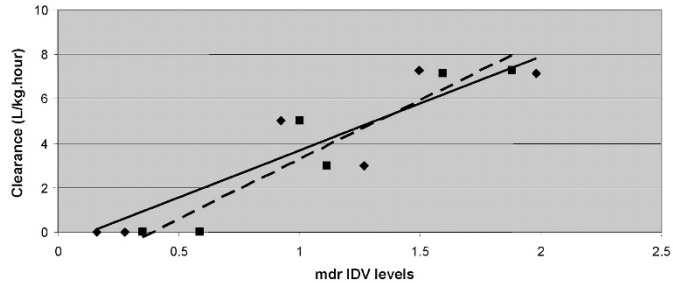

Figure 5. The correlation between digoxin clearance rates and the ontogeny of mdr (mdr1b, $r^2 = 0.83$; mdr1a, $r^2 = 0.89$). Digoxin clearance rates (L/kg.hour) from the pharmacokinetic study were plotted against mdr1b (◆, solid line) and mdr1a (■, dashed line) IDV levels obtained from RT-PCR. Statistically significant correlations between expression of mdr1b and mdr1a and the clearance rate of digoxin were observed.

the age groups had levels of mdr1a that were different from adults. Levels of mdr1b on the other hand, were all significantly different from adult, which the authors concluded indicates a steady, more gradual rise in expression. Contrary to these studies, Mahmood *et al.* found no evidence for postnatal changes in renal P-gp (13). They used Western blotting to quantitate levels of protein and concluded that at all age groups, protein levels were 96 ± 15% of adult levels.

The differences among these studies might be due to the different animal models and strains used. Dutt *et al.* and Mahmood *et al.* used CF1 and FVB mice, respectively, while Rosati *et al.* used Wistar rats (13,21,22). We used FVB mice in our study, similar to Mahmood *et al.* The use of different techniques could also account for some differences seen. The mRNA expression of mdr1a and mdr1b genes does not necessarily translate into functional P-gp levels at the cell membrane. It is known that P-gp does undergo some posttranscriptional control. Taiplensuu *et al.* studied the power of transcript analysis as a predictor of P-gp phenotype in Caco-2 cell lines (23) and found that measuring *MDR1* transcript alone is at least as valid as measuring the quantity of MDR1 protein in predicting MDR1 functional activity. High correlations were obtained between transcript levels and digoxin efflux. They also found that that digoxin is a specific marker for P-gp-dependent drug efflux (23). Our study is strengthened by the use of both transcript analysis of mdr1b and mdr1a, and the functional clearance of digoxin to elucidate the ontogeny of P-gp.

Our pharmacokinetic data document significant correlation between mdr expression and clearance rates across ages with a higher digoxin clearance rate in mice at Day 21 around the time of weaning, compared with either Day 14 or Day 28. This is consistent with previous studies and clinical experience, which dictates that toddlers require higher doses of digoxin than adults to achieve similar serum concentrations. Nyberg *et al.* first proposed a renal mechanism behind this phenomenon (3). In 1992, Tanigawara and colleagues demonstrated that digoxin is filtered and secreted in the kidney by the renal tubular cells using P-gp (24). Our work provides the first evidence of an ontogeny of renal P-gp in parallel with a similar ontogeny in digoxin clearance rate, with higher RNA expression during the time of weaning.

Of importance, for a number of other renally excreted drugs, there is now clinical evidence from a number of pharmacokinetic studies of higher clearance rates and dose requirements in toddlers after the postnatal period, typically higher than adult dosage requirements (25). To the best of our knowledge, the present work is the first to explore this phenomenon as a result of transporter ontogeny.

The renal handling of digoxin is possibly mediated also by other active processes, not only P-gp. Oatp2, a member of the organic anion transporting polypeptide (Oatp) family cloned from rat liver, was also shown to transport digoxin (26). However, some species-specific differences exist between rats and humans and expression in the kidney is minimal. More promising is the recent discovery of the first member of the Oatp family to be localized predominantly in the kidney, on the basolateral membrane of renal proximal tubules (27).

A second hypothesis tested in the present study was that the enhanced clearance rate at the time of weaning might be due to larger kidneys in proportion to body weight. Yet through direct measurements of body and kidney weight this hypothesis was rejected, as the ratio of kidney weight to body weight remained constant. We acknowledge that proportionally larger kidneys based on weight do not address the number of nephrons present; however existing work shows no increase in the number of nephrons after birth (28). We also recognize that the renal clearance of digoxin is a sum of its glomerular filtration and net tubular secretion. However, there is strong evidence of a lack of ontogeny in GFR (29,30,31).

There are limitations to cross-species extrapolation of results documented in mice. The fact remains that mice have two genes encoding the drug-transporting P-gp, whereas humans have one. Some evidence suggests that the mdr1a gene product is a more efficient efflux pump, (32) while endogenous P-gp regulators like progesterone inhibit drug efflux with more potency in cell lines overexpressing mdr1b (33). However, much of what is known about the renal handling of digoxin and other drugs by murine P-gp is similar to its human correlate, (34) even in their drug-drug interactions (15). The less statistically strong differences shown by us for mdr1a may reflect a limited statistical power, or a genuine difference from mdr1b. This question will need to be answered by future investigations. The robustness of our results presented here will only be strengthened once this study has been repeated using pediatric tissue.

CONCLUSION

It has become increasingly clear to clinicians that children cannot be treated as smaller adults in the way their kidneys handle drugs. There are both qualitative and quantitative difference in pharmacokinetics and pharmacodynamics. Our experimental strategy used parallel *in vitro* and *in vivo* methods to help uncover developmental changes in renal drug disposition. It is the first in our knowledge to link the ontogeny of a renal drug transporter with age-dependant maturation of clearance. We found there is an ontogeny of mdr1b expression during the weaning period in mice, with a parallel faster digoxin clearance rate. This may point to the mechanism behind why young children require higher doses of digoxin. Our work has yielded new information that may have important implications for pediatric dosing, and improved safety of drug therapy in children.

Acknowledgments. We would like to thank Christopher Paciorek for technical assistance and the staff of the Laboratory Animal Services for their care of animals used in this study. NP was awarded the American Society for Clinical Pharmacology and Therapeutics' Presidential Trainee Award for this work (Miami, Florida, March 2004).

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