Generation and Characterization of a Novel Multidrug Resistance Protein 2 Humanized Mouse Line

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ABSTRACT:
The multidrug resistance protein (MRP) 2 is predominantly expressed in liver, intestine, and kidney, where it plays an important role in the excretion of a range of drugs and their metabolites or endogenous compounds into bile, feces, and urine. Mrp knockout [Mrp2(−/−)] mice have been used recently to study the role of MRP2 in drug disposition. Here, we describe the first generation and initial characterization of a mouse line humanized for MRP2 (huMRP2), which is nulled for the mouse Mrp2 gene and expresses the human transporter in the organs and cell types where MRP2 is normally expressed. Analysis of the mRNA expression for selected cytochrome P450 and transporter genes revealed no major changes in huMRP2 mice compared with wild-type controls. We show that human MRP2 is able to compensate functionally for the loss of the mouse transporter as demonstrated by comparable bilirubin levels in the humanized mice and wild-type controls, in contrast to the hyperbilirubinemia phenotype that is observed in MRP2(−/−) mice. The huMRP2 mouse provides a model to study the role of the human transporter in drug disposition and in assessing the in vivo consequences of inhibiting this transporter by compounds interacting with human MRP2.

Introduction
The multidrug resistance protein (MRP) 2, also referred to as ABCC2 or cMOAT, is a member of the superfamily of ABC transporters, which constitutes a large class of membrane transporters, receptors, and ion channels (reviewed in Stanley et al., 2009). Like other members of this family, for example P-glycoprotein (MDR1) or breast cancer resistance protein, MRP2 has an important function in the transport of various drugs and their metabolites (Chan et al., 2004; Leslie et al., 2005). Accordingly, the transporter is expressed at many different sites of drug metabolism, such as the liver, intestine, or kidney, where it is located in the apical membranes of hepatocytes, mucosal cells, and proximal tubular cells (Leslie et al., 2001, 2005). Because of its location in these tissues, MRP2 facilitates the excretion of substrates into bile, feces, and urine, and thus limits the xenobiotic accumulation in the body (Fricker and Miller, 2002). The expression of MRP2 in different solid human tumors and its capacity to transport many anticancer drugs is clinically important because it can contribute to anticancer drug resistance (Sandusky et al., 2002; Haimeur et al., 2004; Surowiak et al., 2006). In addition to its role in xenobiotic disposition, MRP2 also plays a role in the transport of endogenous compounds, in particular the secretion of bilirubin glucuronides into the bile. A deficiency of this transporter in humans caused by mutations in the MRP2 gene gives rise to the inherited disorder Dubin-Johnson syndrome, associated with conjugated hyperbilirubinemia and pigment deposition in the liver (Zimniak, 1993), and comparable effects were observed in naturally occurring MRP2-deficient rat strains (Jansen et al., 1985; Hosokawa et al., 1992; Paulusma et al., 1999). Because of its importance in the excretion of xenobiotics or their metabolites and in bilirubin homeostasis, drug-mediated inhibition of MRP2 can be a significant safety issue for drugs in development (Horikawa et al., 2002).

Two viable and fertile Mrp2 knockout [Mrp2(−/−)] mouse lines with a mild hyperbilirubinemia phenotype have been reported by targeted gene disruption (Chu et al., 2006; Vlaming et al., 2006). Studies on these knockout lines demonstrated that the murine MRP2 restricts the plasma levels of the MRP2 probe substrate dibromosulfophthalein and two heterocyclic amine dietary carcinogens and significantly contributes to the elimination of the anticancer drug methotrexate from the blood in a dose-dependent manner (Chu et al., 2006; Vlaming et al., 2006).

The MRP2(−/−) mouse lines have been shown to be valuable models for establishing the role of MRP2 in drug disposition. Provided that a compound is transported equally by the mouse and the human transporter, differences in the clearance between wild-type

ABBREVIATIONS: MRP, multidrug resistance protein; ABC, ATP-binding cassette; WT, wild type; kb, kilobase; PCR, polymerase chain reaction; ES, embryonic stem; huMRP, humanized multidrug resistance protein; PXR, pregnane X receptor; huPXR, humanized PXR; RT, reverse transcription; Ct, cycle threshold; BSA, bovine serum albumin; HRP, horseradish peroxidase.

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(WT) and knockout mouse lines can help to assess the relevance of MRP2 in drug disposition in man. For example, there is a report that the heterozygous loss of function in the MRP2 gene caused the impaired elimination of methotrexate in a patient, resulting in severe overdosing and nephrotoxicity (Hulot et al., 2005). Such an effect is in agreement with the role of MRP2 in mice, as described above (Vlaming et al., 2006). However, significant species differences in the transport efficiency of MRP2 substrates and in the modulation of transport by other compounds have been described between rodents and humans. For example, studies using Madin-Darby canine kidney cells expressing the human or mouse transporter showed that saquinavir and docetaxel were more efficiently transported by mouse MRP2, whereas human MRP2 was a more effective transporter of vinblastine. For several drugs, the stimulation of MRP2-mediated transport by probenecid and sulfinpyrazone was more pronounced for the human than the mouse transporter, and the modulating effect of sulfinpyrazone on both transporters was, in fact, opposite (Zimmermann et al., 2008).

In light of the species differences, we have for the first time generated a mouse humanized for MRP2. This was achieved by a knock-in strategy allowing the expression of the human transporter under control of the corresponding mouse promoter. The expression of the endogenous mouse MRP2 gene was disrupted by this approach. We show that the human transporter was expressed at the predicted sites of drug disposition, such as the liver, intestine, and kidney, with a correct cellular and subcellular localization. By determining plasma bilirubin levels, we provide evidence that the human MRP2 protein is active and able to reverse the hyperbilirubinemia observed in MRP2-null animals.

### Materials and Methods

**Animal Husbandry.** Mice were kept in Tecniplast (Hohenpeissenberg, Germany) Sealsafe microisolator cages. Food and water were available ad libitum. Light cycles were on a 13-/11-h light/dark cycle, with the light phasing starting at 6:00 AM. Temperature and relative humidity were maintained between 21 and 23°C, and 45 and 65%, respectively.

**Construction of the MRP2 Targeting Vector.** A 6.2-kilobase (kb) genomic fragment comprising 4.4 kb sequence upstream of the translational start site, exon 1, intron 1, and exon 2 of the mouse MRP2 gene (5’ targeting arm) and a 2.6-kb genomic fragment downstream from exon 2 (3’ targeting arm) were subcloned by red/ET recombineering (Zhang et al., 1998) and used as targeting arms for homologous recombination. The 6.2-kb 5’ targeting arm and a cDNA comprising exons 2 to 32 of human MRP2 were linked by subcloning, and by polymerase chain reaction (PCR) the human cDNA was fused to the mouse genomic sequence such that exons 2 to 32 of human MRP2 replaced exon 2 of the mouse gene. The fused fragment of 5’ targeting arm and partial human cDNA were subcloned into a predesigned vector carrying the 3’ targeting arm, a synthetic polyA signal, an frt-flanked neomycin expression cassette, and a thymidine kinase expression cassette, to give rise to the targeting vector depicted in Fig. 1A.

**Generation and Molecular Characterization of Targeted Embryonic Stem Cells.** Culture and targeted mutagenesis of embryonic stem (ES) cells were carried out as described previously (Hogan et al., 1994). The targeting vector was linearized with NotI and electroporated into a C57BL/6 mouse ES cell line. Of 456 G418- and Gancyclovir-resistant ES cell colonies screened by standard Southern blot analyzes, two correctly targeted clones were identified. Expansion and further analysis of both clones by Southern blot analyzes with different suitable restriction enzymes and 5’ and 3’ external probes and an internal neomycin probe confirmed that these clones were correctly targeted at both homology arms and did not carry additional random integrations (data not shown).

Generation and Molecular Characterization of MRP2-Humanized Mice. One of the MRP2-targeted ES cell clones described above was expanded, injected into BALBc-blastocysts, and transferred into foster mothers as described previously (Hogan et al., 1994). Litters from these fosteres were inspected visually, and chimerism was determined by hair color. Highly chimeric animals were used for further breeding in a C57BL/6 genetic background. The neomycin selection cassette was removed in vivo by crossing to an efficient FLP-deleter (FLP-deleter) strain carrying a transgene that expresses FLPe in the germline (Fig. 1A).

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**Figure 1.** Strategy to generate huMRP2 mice. A, a partial cDNA of human MRP2 comprising exons 2 to 32 followed by a polyA motif was used to replace exon 2 of mouse MRP2 by homologous recombination. Mouse exons are represented as black bars, the human cDNA is represented as a dotted arrow, and the targeting arms are represented as dark and light gray lines. The sequence of mouse exon 1, potentially coding for the leader sequence, is retained so that a fusion protein of mouse leader sequence and amino acids encoded by human exons 2 to 32 is expressed. Targeted mice are crossed to a mouse strain expressing the FLPe recombinase to delete the neomycin selection cassette by FLP-medi- ated recombination at the frit-sites (black triangles) and to generate MRP2-human- ized mice. For the sake of clarity, se- quences of the targeting vector are not drawn to scale. Neo, neomycin expression cassette; TK, thymidine expression cassette. B, comparison of the amino acids encoded by mouse and human exon 1. The human sequence differs from the mouse sequence at two positions: an aspartic acid to leucine exchange at position 2 and an additional lysine at position 4.
The FLPe-deleter had been generated in-house on a C57BL/6 genetic background. The MRP2-humanized allele was detected by PCR with the primers 5′-GAGAATGTTGGAAGAAGG-3′ and 5′-TCAGCAAAAACAGGACC-3′, giving rise to a 387-basepair fragment, whereas a 188-basepair fragment obtained with the primers 5′-GAGAATGTTGGGAAAGG-3′ and 5′-TCTGTAAATGCGCAAGGC-3′ indicates the presence of the MRP2 WT allele. MRP2(+/-) mice (Vlaming et al., 2006) were obtained from Taconic Farms (model number 000621-M; Germantown, NY). MRP2/PXR double humanized mice were generated by crossing huMRP2 animals with the humanized pregnane X receptor (huPXR) mice as described recently (Scheer et al., 2008). Homozygously humanized and knockout mice for each locus were used for all studies. WT C57BL/6J and FVB animals purchased from either Harlan (Shardlow, UK) or Taconic Farms were used for control experiments.

Animal and Treatments. All animal procedures were carried out under a United Kingdom Home Office license, and all animal studies were approved by the Ethical Review Committee, University of Dundee. Male, sexually mature MRP2(+/-), huMRP2, huMRP2/huPXR, WT C57BL/6J, or FVB mice were used for all experiments.

Quantitative Reverse Transcription-PCR. mRNA expression was analyzed by quantitative reverse transcription (RT)-PCR (TaqMan). Total RNA was prepared from small intestine using TRIzol reagent (Invitrogen, Carlsbad, CA) and from the liver and kidney using the QiAgen RNA mini kit (QiAgen, Hilden, Germany). cDNA was synthesized from 1 μg of total RNA using the Quantscript Reverse Transciptase Kit (QiAgen). Primers were used from the following Assay-On-Demand Kits: mouse MRP2, Mm00496898_m1; human MRP2, Hs00166123_m1; mouse Cyp2b10, Mm00456588_mH; mouse Cyp2f5, Mm00472168_m1; mouse Mdr1a, Mm01234133_m1; mouse Mdr1b, Mm00440736_m1; mouse MRP3, Mm00551550_m1; mouse MRP4, Mm01226381_m1; and mouse β-actin, Mm00607939_m1 (Applied Biosystems, Foster City, CA). Quantitative RT-PCRs were performed using TaqMan Universal PCRMastermix in an ABI PRISM 7000 Sequence Detection System (Applied Biosystems). Data were analyzed using comparative cycling time methodology, in which fluorescent output, measured as cycle threshold (Ct), was directly proportional to input cDNA concentration. A Ct value of 40 was interpreted as absence of gene expression, whereas Ct values in the range of 35 to 40 were interpreted as being at the limit of detection of the TaqMan and, therefore, were not quantitatively analyzed. Input cDNA concentrations were normalized to murine β-actin. Calculations were performed by a comparative method (2^(-ΔΔCt)).

Sequencing Analysis. RT-PCR was performed using RNA isolated from a huMRP2 mouse using oligos to amplify the potential full-length transcript, and the 5′- and 3′-half of the MRP2 cDNA fragments, respectively. The oligos used were as follows: 5′-gggatcctgacctagctac-tc-3′ (MRP2_F2), 5′-agggagacccagctacctgac-tc-3′ (MRP2_F3), 5′-ctctgaggctctcttgctctgtctgagctc-3′ (MRP2_R2), and 5′-CATCTGACGCCCCCTGCTGGACTT-3′ (MRP2_R3). One-step RT-PCR was conducted using Superscript RT-PCR Kit (Invitrogen). Sequence analysis was performed by Lark Technologies Ltd (Takeley, UK). Alignments were performed using Tcoffee (http://tcoffee.vital-it.ch/cgi-bin/Tcoffee/tcoffee.cgi/index.cgi) and Contig express and Align-X programs of Vector NTI Software (Invitrogen).

Immunohistochemistry. Liver, kidney, and duodenum sections were processed, paraffin wax-embedded, then sectioned. All sections were cut at 5 μm using a standard microtome. A mouse monoclonal antibody specific to human MRP2 (at a dilution of 1:100; Abcam) was used for immunohistochemical detection of human MRP2 protein liver and kidney: Antigen Unmasking Solution (Vector Laboratories, Peterborough, UK) was used. The antigen retrieval using Toshiba (Tokyo, Japan) microwave X-231 was modified for this step as follows. The Antigen Unmasking Solution was preheated for 3 min at full power in the microwave, and then the plastic rack (containing the slides) was placed in the trough and heated for another 5 min at full power. Afterward, the trough was incubated for 10 min at the lowest power setting. For small intestine, EDTA (1 mM, pH 8.0) was used. EDTA was preheated for 3 min at full power using a Toshiba microwave X-231. The slides were placed into the hot EDTA, and then liquid was simmered for 15 min at the lowest power setting on the microwave. Normal goat serum [diluted to 5% in TBST (50 mM Tris (pH7.6)/15 mM NaCl/0.05% Tween-20)] Vector Laboratories) and a peroxidase block (DakoCytomation Ltd.) were used to block nonspecific binding of the primary and secondary antibodies and to quench endogenous peroxidase activity. Mouse EnVision kits (DakoCytomation Ltd.) were used to detect bound primary antibodies. DakoCytomation DAB+ was used as the staining agent and was used according to manufacturer’s guidelines. Stained slides were dehydrated and mounted using DPX.

DakoCytomation EnVision+ Dual Link System-HRP (DAB+) was used to visualize MRP2 expression, and all slides were counterstained with hematoxylin. Images were captured using a Carl Zeiss Axio Imager A1 microscope (Carl Zeiss, Jena, Germany), along with Velocity 4 software (Velocity Software, Mountain View, CA).

Analysis of Bilirubin Conjugates and Total Bilirubin. Terminal-conjugated bilirubin (bilirubin-direct) and total bilirubin (bilirubin-total), containing both conjugated and unconjugated forms, were analyzed using BIL-D and BIL-T analyte cassettes (Roche Diagnostics, Basel, Switzerland) on the CO-BAS Integra 400+ (Roche Diagnostics).

Statistics. Statistical significance was assessed to determine differences after drug treatment between mouse lines using a two-tailed, paired, Student’s t test. The criterion for statistical significance was P < 0.05.

Results

Humanization of Mice for MRP2 by Knock-in into the Endogenous Gene Locus. MRP2-humanized mice were generated by a knock-in strategy (as depicted in Fig. 1A) such that a partial cDNA of human MRP2 comprising exons 2 to 32 followed by a polyA motif was used to replace exon 2 of mouse MRP2 by homologous recombination. This approach retains mouse exon 1 so that instead of the mouse transporter, a fusion protein of amino acids encoded by mouse exon 1 and human exons 2 to 32 is expressed under control of the mouse MRP2 promoter. Because the first amino acids often code for the leader sequence, which is required for the proper localization of the protein within the cell, this strategy ensures an accurate allocation of the transporter protein by the mouse trafficking machinery. The amino acid sequence encoded by mouse and human exon 1 are different at two positions, with an aspartic acid (mouse) to leucine (human) change at position 2 and an additional lysine at position 4 of the human protein (Fig. 1B). It should also be noted that mouse and human exon 2 start with the same three base pairs. Therefore, the splice site at the intron 1/exon 2 junction is retained, allowing an efficient splicing of mouse exon 1 to the human exon 2 to 32 cDNA in the huMRP2 mice.

Homozygous huMRP2 mice appeared normal, could not be distinguished from WT animals, and had normal survival rates and fertility.
Hematoxylin and eosin analysis on livers of different transgenic animals \((n = 5)\) revealed that the microscopic findings recorded were indicative of normal histology (data not shown).

**Stable Expression of Human MRP2 in Liver, Kidney, and Intestine of Humanized Mice.** Quantitative PCR analysis was carried out to demonstrate that human, but not mouse, MRP2 mRNA is expressed in the humanized mice. Mouse MRP2 mRNA was expressed in the kidney, liver, and intestine of WT, but not in humanized mice, whereas the human MRP2 mRNA but not the mouse MRP2 mRNA was expressed in these tissues in the humanized animals (data not shown). A direct comparison of the expression levels of human MRP2 mRNA in the huMRP2 mouse line relative to murine MRP2 mRNA in WT animals is shown on Fig. 2. Expression levels of the human transcript in the kidney, liver, and duodenum of the huMRP2 mouse line were increased by 2.0-, 3.7-, and 2.2-fold, respectively, compared with the expression of mouse MRP2 mRNA in WT controls \((n = 3\) mice for all tissues and each mouse line). The reason for the higher expression of the human transcript in the humanized mice and how this relates to quantitative differences in protein expression currently is not known.

To further assess whether the expression of the human MRP2 mRNA is translated into protein, Western blot and immunohistochemistry measurements were performed. Using an antibody specific to the human MRP2 protein, expression was detected in the liver, kidney, and intestine of huMRP2 mice by Western blot analysis (Fig. 3A, right). The specificity of the antibody was confirmed by the lack of a signal in samples from WT and Mrp2\((-/-)\) mice. We also tested for the absence of the mouse MRP2 transporter in the humanized mice with an antibody specific for the murine protein. In agreement with the TaqMan data, mouse MRP2 is only expressed in WT mice, whereas no expression is observed in huMRP2 or Mrp2\((-/-)\) animals (Fig. 3A, left).

Because a correct cellular expression in the different organs is critical for the correct MRP2 function, we then used the human-specific MRP2 antibody in immunohistochemical analysis in liver, kidney, and intestine (Fig. 3B, bottom). In the liver, expression was observed in the perportal region with small spots and irregularly shaped elongated structures between adjacent hepatocytes. This indicates expression of the human transporter in bile canalicular structures where MRP2 is normally expressed (Faber et al., 2003). At low magnification \((100\times)\) immunoreactivity was detected in the cortex but not in the papillae of the kidney, and at high magnification \((400\times)\) staining was observed at the luminal membrane of the renal proximal tubules. This is again in agreement with the expected localization profile of MRP2 (Schaub et al., 1997, 1999). Finally, in the duodenum, MRP2 was expressed in the luminal (apical) membrane of...
enterocytes at the surface of the villi (Mottino et al., 2000). With the human-specific MRP2 antibody, no staining in any of the three tissues tested was observed in the WT (Fig. 3B, top) and MRP(−/−) mice (data not shown).

**Expression Profile of Selected Genes in huMRP2 Compared with WT Mice.** To assess potential compensatory gene expression changes in the kidney, liver, or duodenum of huMRP2, we compared the mRNA levels of selected cytochrome P450 and transporter genes with WT Mice.

To assess potential compensatory gene expression changes in the kidney, liver, or duodenum of huMRP2, we compared the mRNA levels of selected cytochrome P450 and transporter genes with WT Mice. For this limited analysis, we chose Cyp2b10, Cyp2c55, Mdr1a, Mdr1b, Mrp3, and Mrp4. The two cytochrome P450s were selected because of the altered levels previously described in a Cyp3a knockout mouse (van Waterschoot et al., 2009), and the transporters were selected because of the overlap in function, tissue distribution, and substrate specificity with MRP2. MRP4 mRNA has been reported to be induced 6-fold in liver and 2.5-fold in kidney of MRP2(−/−) mice, whereas only minor expression changes were observed for other genes (Chu et al., 2006). In kidney, liver, and duodenum of huMRP2 mice, all changes in expression of the six genes analyzed were minor and statistically not significant (Table 1). In particular, the expression of MRP4 was not changed compared with WT controls.

**Bilirubin Levels in the Urine and Plasma of WT, MRP2(−/−), and huMRP2 Mice.** The hemoglobin breakdown product bilirubin is a known high-affinity physiological substrate of MRP2 (Chu et al., 2006; Vlaming et al., 2006), and a deficiency of this transporter is associated with conjugated hyperbilirubinemia in man (Zinniak, 1993), rats (Jansen et al., 1985; Hosokawa et al., 1992; Paulusma et al., 1999), and mice (Chu et al., 2006; Vlaming et al., 2006). To investigate the functionality of human MRp2 in the huMRP2 mouse line, total and conjugated bilirubin levels were initially analyzed in the serum and urine of WT and huMRP2 mice. A first indication of reversion of the hyperbilirubinemia phenotype described for the MRP2(−/−) mice in the huMRP2 model was obtained by comparing bilirubin levels in C57BL/6 WT animals and huMRP2 mice. No significant changes in conjugated or total (unconjugated and conjugated) bilirubin levels in either urine or serum could be observed between WT and huMRP2 mice (n = 3 per mouse line; Supplemental Table 1).

For practical reasons, the most extensive assessment of bilirubin levels was carried out in double homozygous offspring from a cross of the huMRP2 model with a mouse line humanized for the PXR. The huPXR mouse line was described recently (Scheer et al., 2008). More importantly, no changes in total or conjugated bilirubin were observed in the huPXR model compared with WT mice. Furthermore, based on quantitative PCR analysis and immunohistochemistry, human MRP2 mRNA and protein levels were comparable between huMRP2 and huMRP2/huPXR mice (data not shown). Because the available MRP2(−/−) mouse line has a different genetic background (FVB) than both the huMRP2 mice and the derived huMRP2/huPXR model (C57BL/6), we included both FVB and C57BL/6 WT animals in this study because of potential differences in the bilirubin levels between the two mouse strains. Plasma bilirubin conjugates were markedly increased (by 93-fold) in the MRP2(−/−) mice compared with the corresponding FVB WT control (Fig. 4A). In addition, total bilirubin was also statistically increased significantly in the null strain by 4-fold (Fig. 4B). In contrast, both conjugated and total plasma bilirubin levels were indistinguishable between the huMRP2/huPXR and the corresponding C57BL/6 WT control. Slightly higher conjugated and total plasma bilirubin levels were measured in C57BL/6 mice compared with FVB animals. Total and conjugated bilirubin levels in urine were statistically increased significantly by 5- and 3-fold in the MRP2(−/−) mice compared with FVB WT controls, respectively, whereas no significant difference was observed between the huMRP2/huPXR mice and the C57BL/6 WT animals (Fig. 4, C and D). The urine bilirubin levels were moderately higher in C57BL/6 than in FVB WT mice.

In conclusion, the data demonstrate that with regard to transport of bilirubin and its conjugates, human MRP2 is able to compensate for the loss of the mouse transporter in both huMRP2 and huMRP2/huPXR mice.

**Discussion**

Membrane transporters are increasingly recognized as major determinants of the pharmacokinetics, safety, and efficacy profiles of drugs (Giacomini et al., 2010). Although a variety of in vitro assays to study the role of transporters in drug disposition or drug-drug interactions have been developed, it is sometimes difficult to extrapolate these data to the in vivo situation, particularly in relation to tissue distribution (Balimane et al., 2006). In addition, species differences in the function of drug transporters limit the use of traditional animal models for studying such interactions. To overcome this limitation, we and others

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**TABLE 1**

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<th>Gene</th>
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<th>Duodenum</th>
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<td>0.7 ± 0.1</td>
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<td>Mrp4</td>
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*Low gene expression Ct >32.
have generated mouse models expressing human instead of mouse transporters. Humanized mouse models for the organic anion-transporting polypeptides OATP1B1 and OATP1B3 have been developed recently (van de Steeg et al., 2009, 2012). Apart from these, no humanized transporter mice have been described to date.

In this report, we describe the generation and characterization of a mouse line humanized for MRP2. These mice were healthy, had normal survival rates and fertility, and did not show any overt phenotype. The human MRP2 transcript was expressed in the organs known to express this transporter, such as the kidney, the liver, and the intestine. The expression of the human mRNA in huMRP2 mice was 2.0-, 3.7-, and 2.2-fold higher in kidney, liver, and duodenum compared with mouse MRP2 expression in WT controls. Immunohistochemistry demonstrated the correct cellular localization of the human MRP2 protein in the different organs. Accordingly, the human transporter can be detected in bile canalicular structures in the perportal regions of the liver, in the luminal membrane of the proximal tubules in the cortex of the kidney, and in the luminal membrane of enterocytes at the surface of the villi from the duodenum.

The expression of Cyp2b10, Cypc5, Mdr1a, Mdr1b, Mrp3, and Mrp4 mRNA in kidney, liver, and duodenum of huMRP2 mice was unchanged compared with WT controls, demonstrating the lack of compensatory changes for these selected genes. This is in an agreement with the finding that only minor changes in the expression of 98 analyzed phase I, phase II, and transporter genes were observed in MRP2(−/−) mice (Chu et al., 2006). It is interesting to note that MRP4 expression was significantly increased in kidney and liver of the MRP2 knockout, but it was not changed in huMRP2 mice. Therefore, it seems that human MRP2 compensates for the loss of the mouse transporter.

To demonstrate functional human MRP2 transporter expression in the huMRP2 mouse line, we compared the level of total and conjugated bilirubin in the plasma and urine of WT, Mrp2(−/−), and huMRP2 mice, as well as in a derived mouse model double humanized for MRP2 and PXR (huMRP2/huPXR). Although a deficiency in MRP2 is associated with hyperbilirubinemia in humans, rats, and mice (Jansen et al., 1985; Hosokawa et al., 1992; Zimniak, 1993; Paulusma et al., 1999; Chu et al., 2006; Vlationg et al., 2006), bilirubin levels in both huMRP2 and huMRP2/huPXR mice were indistinguishable from those in WT animals, demonstrating that the human MRP2 is functionally active.

MRP2 plays an important role in conferring resistance to multiple chemotherapeutic agents (Kruh et al., 2001; Haineur et al., 2004; Surovik et al., 2006; Noma et al., 2008), and species variations in substrate specificity have been identified as a potential limitation in the extrapolation of animal data to man (Leslie et al., 2005; Zimmermann et al., 2008). Several in vitro studies suggest significant differences in the transport efficiency between rodents and humans (Nomiya et al., 2006; Takekuma et al., 2007; Tian et al., 2008; Zimmermann et al., 2008). The substrate specificity differences can be rationalized by protein sequence variation. For example, skate MRP2 protein only shares 56% amino acid identity with its human ortholog (Cai et al., 2003). There is an approximately 80% sequence identity between mouse and human MRP2, so significant amino acid differences do exist. Because single amino acid polymorphisms and even synonymous single nucleotide polymorphisms in human transporters, such as P-glycoprotein and MRP2, can alter significantly the transport efficiency for different substrates (Hoffmeyer et al., 2000; Roberts et al., 2002; Suzuki and Sugiyama, 2002; Kimchi-Sarfaty et al., 2007; Leschziner et al., 2007), the observed differences in the kinetics of MRP2-mediated drug transport between human and mouse are expected. Therefore, it will be interesting to investigate the distribution of anticancer and other drugs in the huMRP2 model. It should be noted that by retaining mouse exon 1 in the huMRP2 model, a fusion protein of amino acids encoded by mouse exon 1 and human exons 2 to 32 is expressed. As a consequence, the extracellular N-terminal part of the transporter contains two amino acid changes compared with the human protein. Because these changes are not in the essential functional domains of the protein, such as the transmembrane domains, ATP-binding domains, or substrate-binding sites (Jedlitschky et al., 2006), it is unlikely that these alterations will have an effect on the functionality or substrate specificity of the human transporter.

Other applications of the huMRP2 mouse are for studying MRP2-mediated drug-drug interactions in vivo and to assess the consequences of MRP2 inhibition on endogenous compounds that are substrates of this transporter. Because MRP2 is a major factor in facilitating the hepatobiliary excretion of bilirubin, the huMRP2 model could provide a human-relevant, in vivo tool for more accurately predicting bilirubinemia in the clinic, particularly for inhibitors that are selective for the human transporter. Urinary coproporphyrin ratios were recently identified as a useful clinical biomarker of MRP2 function (Benz-de Bretagne et al., 2011). It will be interesting to explore whether the analysis of this novel biomarker in the huMRP2 mice might provide a reliable method to predict adverse effects associated with MRP2 inhibition.

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Authorship Contributions

Participated in research design: Scheer, Balilame, Kauselmann, and Wolf.

Conducted experiments: Buechel and Hayward.

Contributed new reagents or analytic tools: Buechel and Hayward.

Performed data analysis: Scheer and Wolf.

Wrote or contributed to the writing of the manuscript: Scheer, Balilame, and Wolf.

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