Regulation of Expression of the Multidrug Resistance-Associated Protein 2 (MRP2) and Its Role in Drug Disposition

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ABSTRACT
The multidrug resistance protein 2 (MRP2; ABCC2) is an ATP-binding cassette transporter accepting a diverse range of substrates, including glutathione, glucuronide, and sulfate conjugates of many endo- and xenobiotics. MRP2 generally performs excretory or protective roles, and it is expressed on the apical domain of hepatocytes, enterocytes of the proximal small intestine, and proximal renal tubular cells, as well as in the brain and the placenta. MRP2 is regulated at several levels, including membrane retrieval and reinsertion, translation, and transcription. In addition to transport of conjugates, MRP2 transports cancer chemotherapeutics, uricosurics, antibiotics, leukotrienes, glutathione, toxins, and heavy metals. Several mutagenesis studies have described critical residues for substrate binding and various naturally occurring mutations that eliminate MRP2 expression or function. MRP2 is important clinically as it modulates the pharmacokinetics of many drugs, and its expression and activity are also altered by certain drugs and disease states.

The identification of the multidrug resistance protein 2 (MRP2; ABCC2) as the transporter that mediates the biliary excretion of numerous drugs and their metabolites has represented a major step forward in understanding the factors that regulate hepatic drug elimination and contribute to hepatic toxicity. MRP2 (ABCC2) is the second member identified in the now nine-member family of MRP membrane transporters (Dean et al., 2001). MRPs represent one branch of the ATP-binding cassette superfamily of transmembrane proteins that use the energy of ATP hydrolysis to translocate their substrates across biological membranes (Borst et al., 1999) (Fig 1A). The founding member, MRP1, was identified as the transporter for organic anions of endogenous and exogenous origin that were conjugated to glutathione, glucuronide, and sulfate, including leukotriene C₄, 2,4-dinitrophenyl-S-glutathione (DNP-SG), and estradiol-17β-D-glucuronide (E₂17G). These same conjugates were identified as substrates of ATP-dependent transport in liver canalicular membranes; however, MRP1 expression in liver was much too low to account for the high-hepatic transport activity. Spontaneous mutant strains of hyperbilirubinemic rats deficient in biliary excretion of bilirubin glucuronides and glucuronide and glutathione conjugates of xenobiotics, the Groningen yellow/transport deficient Wistar rat (GY/TR⁺⁻) and the Eisai hyperbilirubinemic Sprague-Dawley rat (EHBR) were critical to the cloning of the liver homolog of MRP1, termed Mrp2 (Buchler et al., 1996; Paulusma et al., 1996; Ito et al., 1997). Mrp2 is absent in TR⁺⁻ and EHBR rats.

We gratefully acknowledge Public Health Service Grant GM55343 for support of the work from this laboratory cited here and the Reproductive Sciences Training Program (NIH T32 HD07436) for supporting P.M.G. ABBREVIATIONS: MRP, multidrug resistance-associated protein (lower case refers to nonhuman); DNP-SG, 2,4-dinitrophenyl-S-glutathione; E₂17G, estradiol-17β-D-glucuronide; GY/TR⁺⁻, Groningen yellow/transport deficient Wistar rat; EHBR, Eisai hyperbilirubinemic Sprague-Dawley rat; TM, transmembrane domain; MSD, membrane-spanning domains; NBD, nucleotide-binding domain; DJS, Dubin-Johnson syndrome; MDR, multidrug resistance transporter; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine; Bsep, bile salt export pump; PCN, pregnenolone 16α-carbonitrile; ER-8, everted repeat with an 8-base pair spacer; FXR, farnesoid X receptor; CAR, constitutive androstane receptor; PXR, pregnane X receptor; RXR, retinoid X receptor; RAR, retinoic acid receptor; SN-38, 7-ethyl-10-hydroxy camptothecin; BQ-123, cyclo[Leu-o-Trp-o-Asp-L-Pro-o-Val]; MKS71, 3-N-[2-(7-chloroquinolin-2-yl)vinyl][phenyl]-[2-dimethylcarbamoylethylsulfanyl[methylsulfanyl] propionic acid; NEM-SG, N-ethylmaleimide-glutathione; OATP, organic anion transporting polypeptide.
Fig. 1. A, phylogenetic tree showing the human MRP family, redrawn from Borst et al. (1999). SUR, sulfonylurea receptor; CFTR, cystic fibrosis transmembrane regulator. B, proposed membrane topology of Mrp2, adapted and redrawn from Ito et al., (2001d). C, amino acid sequence alignment for human MRP2 (Q92887), MRP1 (P33527), and MRP2 (Q63120), and rat Mrp2 (Q88563), with respective NCBI protein accession numbers. Sequences were aligned using multiple sequence alignments on BCM Search Launcher (http://dot.imgen.bcm.tmc.edu:9331/multi-align/multi-align.html) and formatted using BoxShade 3.21 (http://www.ch.embnet.org/software/BOX_form.html). Approximate locations of putative TMs were obtained from the NCBI protein entries indicated above and may differ from those assumed in the references. Dots indicate mutations in MRP2 or Mrp2 discussed in the text.
due to distinct mutations in the mrp2 gene, both of which create premature termination codons. Cloning of mrp2 has made possible an understanding of its structure function relationships, localization and regulation of expression, and characterization of the defect in patients with the Dubin-Johnson Syndrome (see below).

**Structure and Function of MRP2**

The membrane topology predicted for MRP2 is like that of MRP1 and contains 17 transmembrane (TM) helices, which form three membrane-spanning domains (MSD1, -2, and -3) connected by poorly conserved linker regions (L0 and L1) and highly conserved nucleotide-binding domains (NBD1 and NBD2) (Fig. 1B) (Borst et al., 1999; Konig et al., 1999a). Both the predicted odd number of transmembrane domains and immunofluorescence studies indicate the extracellular localization of the amino terminus (Konig et al., 1999a).

Studies in patients with Dubin-Johnson Syndrome have revealed valuable information about MRP2 genomic organization and the structure and function of MRP2 protein. The classic Dubin-Johnson syndrome (DJS) consists of elevated total and direct bilirubin, increased urinary coproporphyrin I fraction (>80%), and deposition of a dark pigment in the liver (Toh et al., 1999). Patients with DJS may also have a decreased biliary clearance of bromosulfophthalein and some degree of jaundice (Toh et al., 1999). DJS is linked to mutations in the MRP2 gene; these are summarized in Table 1 (Paulusma et al., 1997; Wada et al., 1998; Toh et al., 1999; Ito et al., 2001e; Mor-Cohen et al., 2001). Homozygous mutations lead to classic DJS, whereas heterozygous mutants have moderately elevated urinary coproporphyrin I fraction (~40%) with normal total and direct bilirubin (Toh et al., 1999). Many of these mutations are localized to NBD1 or NBD2. Unlike other mutations, R1150H mutants of the MRP2 protein mature and are properly localized, but transport activity is impaired (Mor-Cohen et al., 2001). Future studies are needed to identify any polymorphisms and their effect on MRP2 function.

Characterization of the substrate recognition/transport sites of MRP2 has been based on the importance of amino acid residues located in the MSD of other ATP-binding cassette transporters, MDR1, and cystic fibrosis transmembrane regulator, and the closely-related family members MRP1 and MRP3. MRP3 was cloned as a homolog of MRP1 and 2 and is highly expressed on the basolateral membrane of rat and human liver under cholestatic and hyperbilirubinemic conditions (Hirohashi et al., 1998; Kiuchi et al., 1998; Konig et al., 1999b). Hydropathy plot analysis has shown that the structures of MRP1 to 3 are very similar. Initial studies showed that the amino terminal MSD1 of MRP1 is nonessential for substrate transport (Bakos et al., 1998), although the linker region connecting MSD1 and MSD2 is essential for MRP1 transport of leukotriene C4. Likewise, no mutations in MSD1 of MRP2 have been identified in patients with DJS, consistent with this region lacking a critical function for MRP2 in humans (Table 1). Efforts have therefore focused on transmembrane segments TM6 to TM17 of MSD2 and MSD3 (Fig. 1, B and C).

To characterize binding sites for typical anionic MRP2 substrates, studies altering charged (especially cationic) amino acids were performed. Ryu et al. (2000) used site-directed mutagenesis to examine the participation of basic residues in TM6 to TM17 of MRP2 in the transport of a fluorescein substrate, glutathione-methylfluorescein. Thirteen basic residues (His, Arg, Lys) in these regions were substituted with alanine; four mutants (K324A in TM6, K483A in TM9, R1210A in TM16 and R1257A in TM17) were all delivered appropriately to the cell surface when expressed in COS-7 cells yet showed decreased efflux of the substrate (Ryu et al., 2000). Similar studies substituting 10 charged amino acids in rat Mrp2 have exploited the differences in substrate specificity between Mrp2, which transports glucuronide, sulfate, and glutathione conjugates with great efficiency versus Mrp3, which efficiently transports glucuronide and sulfate conjugates but not glutathione conjugates (Ito et al., 2001c). Site-directed mutagenesis of Lys to Met (K325M) and Arg to Leu (R586L) in rat Mrp2 markedly reduced transport of DNP-SG and leukotriene C4, without affecting transport capacity of model glucuronide and sulfate conjugates yet increased the affinity for transport of E217G (Ito et al., 2001c). Unlike other MRPs, Mrp3 also transports taurolitholate, a bile acid. Site-directed mutagenesis studies substi-

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Location</th>
<th>Translation</th>
<th>Domain</th>
<th>Phenotype</th>
<th>Reference</th>
</tr>
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<tr>
<td>−24(C-T)</td>
<td>Promoter</td>
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<td>(Wada et al., 1998; Toh et al., 1999)</td>
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<td>(Toh et al., 1999)</td>
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<td>2302/2302(T-C)/wt</td>
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<td>(Ito et al., 2001e)</td>
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UTR, untranslated region; wt, wild type; bp, base pair; UCP1, urinary coproporphyrin fraction 1.
tuting the cationic amino acid Arg\textsuperscript{586} and Arg\textsuperscript{1096} in rat Mrp2 with neutral amino acids (R586L, R586I, R1096L, and R1096M) or a cationic amino acid (R1096K) led to acquisition of taurocholate transport and retention of glucuronide and glutathione conjugate transport by Mrp2 (Ito et al., 2001d). These authors suggest that the presence of Arg at amino acids 586 and 1096 prevents taurocholate transport by Mrp2 (Ito et al., 2001d).

In other recent studies, a highly conserved tryptophan residue, Trp\textsuperscript{1254}, in the last transmembrane segment (TM17) of MSD3 of Mrp1 has been shown to be essential for transport of E\textsubscript{2}17G (Ito et al., 2001b). Mutation of the analogous Trp\textsuperscript{1254} of MRp2 showed this amino acid to be essential for MRp2 transport of methotrexate; nonconservative substitutions (Ala, Cys) eliminated E\textsubscript{2}17G transport, whereas conservative substitutions (Tyr, Phe) were without effect (Ito et al., 2001a). Only the most conservative substitution (W1254Y) retained leukotriene C\textsubscript{4} transport activity. These data indicate not only that Trp\textsuperscript{1254} is essential for MRp2-mediated transport of methotrexate but also demonstrate the presence of more than one substrate binding site in MRp2. The ability of substrates to inhibit and stimulate transport of other substrates, as discussed below, is consistent with this demonstration of distinct transport/binding sites and is a complexity of MRp2 function that will require significantly more attention.

**Localization of MRp2**

Characterization of the distribution of MRp2 mRNA in tissues other than liver showed that MRp2 is also expressed in the apical membranes of the proximal tubule of the kidney and in the apical membrane of the duodenum and jejunum. Mrp2 is expressed in the brush-border membrane domain of segments S\textsubscript{1}, S\textsubscript{2}, and S\textsubscript{3} of proximal tubule epithelia in rat kidney (Schaub et al., 1997) and in proximal tubules of normal human kidney and clear-cell carcinomas, originating from proximal tubule epithelium (Schaub et al., 1999). When expressed in human embryonic kidney cells (HEK293, MRp2 mediates the ATP-dependent transport of p-aminohippurate with a \(K_m\) value of 880 \(\mu\)M (Leier et al., 2000); however, little is known regarding the role of MRp2 in renal secretion of organic anions. In the rat small intestine, Mrp2 expression is concentrated at the tip of the villus, with the highest concentrations seen in the proximal jejunum, with little Mrp2 protein detected in the distal ileum (Mottino et al., 2000). A similar distribution of the phase II conjugating enzymes, UDP-glucuronosyltransferase and glutathione S-transferase, suggests that metabolism and subsequent efflux of the organic anion conjugates act coordinately to decrease the intestinal absorption of food contaminants and drugs that enter the enterocytes via the digestive tract. Functional studies in vivo and in Ussing chambers demonstrate a serosal-to-mucosal flux of DNP-SG and that this flux is significantly decreased in EHBR (Gotoh et al., 2000). In postpartum lactating rats, the intestinal mass is increased, particularly in late lactation, presumably due to the 2- to 3-fold increase in food consumption at this time. Mrp2 protein expression in jejunum (expressed per milligram of brush-border membrane protein) is also increased 2- to 3-fold in late lactation, and this is reflected in increased secretion of DNP-SG in an everted intestinal sac model (Mottino et al., 2001). Increased expression of Mrp2 may be an adaptive mechanism that serves to protect the organism from absorption of dietary toxins at a time when food consump-

**Regulation of Expression**

MRp2 expression is responsive to a number of drug treatments and diseases affecting the liver, particularly cholestatic liver disease. Recent studies indicate that regulation of MRp2 function occurs at least at three distinct levels. These include its endocytic retrieval from the canalicular membrane, its translational regulation, and its transcriptional regulation.

**Endocytic Retrieval.** MRp2 is synthesized in the endoplasmic reticulum, processed in the Golgi and then translocated to the apical plasma membrane, where it must be inserted into the membrane to mediate transport of substrates across the canalicular membrane into bile. Transporter function is regulated by the dynamic endocytic retrieval and exocytic insertion of transporters between the canalicular membrane and an intracellular pool of vesicles (Haussinger et al., 2000; Kipp and Arias, 2000). Canalicular membrane proteins (e.g., Mrp2 and the bile salt export pump (Bsep)) are present in a pericanalicular pool of vesicles that disapper upon treatment of rats with dibutyryl cAMP due to their rapid sorting to the canalicular domain (Boyer and Soroka, 1995; Gatmaitan et al., 1997; Roelofsen et al., 1998). Conversely, Mrp2 and Bsep transport activity is increased in canalicular membranes isolated from livers treated with dibutyryl cAMP; prior administration of colchicine, which disrupts microtubules, blocks these actions of dibutyryl cAMP. Mrp2 and Bsep also colocalize with the transcytotic marker polymer IgA receptor on microtubule-associated transcytotic vesicles, indicating that these proteins must traffic together (Soroka et al., 1999). Treatment of rats with lipopolysaccharide, which induces cholestasis, induces endocytic retrieval of Mrp2 (Dombrowski et al., 2000; Haussinger et al., 2000); this retrieval is reversible if the liver is perfused with hypo-osmotic buffer within 3 h of lipopolysaccharide (Kubitz et al., 1999b). Perfusion of the liver with hyperosmotic buffer also causes rapid retrieval of Mrp2 and Bsep into a subcanaliculart compartment and decreases bile flow; subsequent perfusion with hypo-osmotic buffer induces reinsertion of Mrp2 into the canalicular membrane and restores bile flow (Kubitz et al., 1997) and biliary excretion of DNP-SG, an Mrp2 substrate (Ito et al., 2001c). Phalloidin, the potent
mushroom hepatotoxin and cholestatic agent derived from *Amanita phalloides*, causes a rapid (within 30 min) retrieval of Mrp2 and other canalicular proteins into intracellular sites; retrieval of Mrp2 coincides with decreased bile flow and decreased biliary excretion of leukotriene C₄ (Rost et al., 1999). Finally, E₂17G causes a rapid inhibition of bile flow and retrieval of Mrp2 into intracellular sites; bile flow recoverers spontaneously and is followed by the exocytic insertion of Mrp2 into the canalicular membrane (Mottino et al., 2002).

Pretreatment of rats with dibutyryl cAMP partially attenuates both the inhibition of bile flow and endocytic retrieval of Mrp2 and significantly accelerates the recovery of flow and exocytic insertion of Mrp2 (Mottino et al., 2002). These data indicate that the function of Mrp2 at the canalicular membrane can be regulated over a very short (i.e., minutes) time frame by its rapid endocytic retrieval from and exocytic insertion into the canalicular membrane. It follows that a breakdown in this process could lead to a decreased amount or absence of Mrp2 (and other transporters) in the canalicular membrane, and hence, cholestasis (Kipp and Arias, 2000).

**Translational Regulation.** Treatment of rats with ethynylestradiol markedly decreases Mrp2 protein expression but has no effect on Mrp2 mRNA expression, effecting neither total Mrp2 mRNA nor the relative abundance of the three Mrp2 mRNA transcripts that contain differing 5′-untranslated regions (Trauner et al., 1997). Similarly, expression of hepatic Mrp2 mRNA is unchanged, whereas expression of Mrp2 protein is decreased by 50% in pregnant versus control rats (Cao et al., 2001), indicative of posttranscriptional control of Mrp2. Conversely, treatment of rats with pregnenolone-16α-carbonitrile (PCN) increases Mrp2 protein expression markedly but has no effect on Mrp2 mRNA expression (Vore et al., 2001; Johnson et al., 2002). To assess the role of translational regulation of Mrp2, we examined the polysomal distribution of Mrp2 mRNA in livers from control and pregnant rats and rats treated with PCN or vehicle (corn oil).

Polysomal distribution analysis entails separation of polyribosomes from single ribosomes and their subunits by differential sedimentation through a sucrose gradient. If the protein encoded by a specific DNA sequence is being actively synthesized, then its mRNA will be associated with a number of ribosomes (polysomes) and will sediment near the bottom of the gradient. We found significantly less Mrp2 mRNA present in the polysomal fraction near the bottom of the gradient in pregnant rats versus controls and a significant increase in this fraction in PCN-treated rats versus Corn-oil controls (Vore et al., 2001). These data show that Mrp2 protein is not being as actively synthesized in livers from pregnant rats, whereas it is being very actively synthesized in livers from PCN-treated rats.

Analysis of the 5′-untranslated region of human MR2 cDNA has identified three possible transcription start sites at −246, −204, and −99 base pairs, with the most common being 246 base pairs 5′ to the ATG translation start codon (Kauffmann and Schrenk, 1998; Tanaka et al., 1999); we identified three such sites for rat Mrp2, at positions −213, −163, and −71 base pairs (Li and Vore, 2002). When each of the three rat and human 5′-untranslated regions were cloned into the pGL3 basic luciferase expression vector and transfected into HepG2 cells, the intermediate and long 5′-untranslated regions of both rat and human MR2 significantly decreased luciferase expression (Li and Vore, 2002). Although these data strongly suggest that the 5′-untranslated region contains sites that decrease the rate of translation of MR2, further studies are needed to identify the mechanism(s) of translational regulation.

**Transcriptional Control.** Transcriptional regulation of MR2 expression has been characterized both with respect to decreased expression in disease and increased expression by treatment with various classic enzyme inducers. Activation of hepatic inflammation by conditions such as sepsis, alcoholic, autoimmune, and viral hepatitis, and parenteral nutrition-associated liver disease is associated with cholestasis and hyperbilirubinemia (Hill et al., 1997). The liver is a principal target of inflammatory mediators, such as tumor necrosis factor-α and interleukin-6 and -1β, and regulates changes in hepatic protein synthesis during the acute phase response. Denson et al. (2000) have identified an RXRα/RARα response element in the Mrp2 promoter and have shown that transcriptional suppression of Mrp2 by acute phase proteins occurs via interleukin-1β-induced reduction in nuclear RXR/RAR heterodimers.

Characterization of increased transcriptional expression of Mrp2 has proven to be more complex. The promoter regions of the human MR2 and the rat Mr2 gene contain a number of putative consensus binding sites for AP1, SP1, HNF1, and HNF3β (Kauffmann and Schrenk, 1998; Tanaka et al., 1999). The −431 to −258 region also contains important elements that control expression in HepG2 cells, particularly the CCAAT-enhancer binding protein β. In primary cultures of rat hepatocytes, dexamethasone, 2-acetylaminofluorene, cisplatin, cycloheximide, phenobarbital, clotrimazole, and PCN all increase Mrp2 mRNA and protein within 24 h (Kauffmann et al., 1997; Courtois et al., 1999; Kubitz et al., 1999a,b). Courtois et al. (1999) further showed that mifepristone did not inhibit Mrp2-induction by dexamethasone in cultured rat hepatocytes, eliminating a glucocorticoid receptor-mediated induction, and that treatment of rats in vivo with dexamethasone also induced Mrp2 mRNA. The inducibility of Mrp2 gene expression in primate liver was investigated in rhesus monkeys treated with tamoxifen or rifampin (Kauffmann et al., 1998). Both tamoxifen and rifampin strongly induced Mrp2 mRNA in two male and two female rhesus; tamoxifen induced Mrp2 protein in both male and female rhesus, whereas rifampin showed some inducing effect in a female but was inactive in a male monkey.

Recent studies demonstrated that ligands for FXR, PXR, and CAR (chenodeoxycholic acid, PCN, dexamethasone, and phenobarbital) all induced Mrp2 mRNA in primary cultures of rat hepatocytes and characterized a putative ER-8 at −401 to −376 of the rat Mrp2 promoter that bound the corresponding FXR/RXR, PXR/RXR, and CAR/RXR heterodimers (Kast et al., 2002). Cotransfection of HepG2 cells with the Mrp2 proximal promoter (−1034 to −15) with plasmids encoding PXR, CAR, or FXR and RXR followed by treatment with receptor-specific ligands led to ligand-specific activation of PXR and FXR, whereas CAR was constitutively active, as expected. When HepG2 cells were cotransfected with pTk-2xER-8, containing two copies of the wild-type ER-8, the reporter was activated over 100-fold in a PXR- and PCN-dependent manner. However, treatment of rats in vivo with phenobarbital leads to inconsistent effects on Mrp2 expression. Thus, Hagenbuch et al. (2001) were unable to show a significant effect of phenobarbital treatment on Mrp2 expres-
sion, consistent with the work of others (Fernandez-Checa et al., 1993; Kiuchi et al., 1998; Ogawa et al., 2000) showing a lack of effect on ATP-dependent transport of Mrp2 substrates (e.g., leukotriene C$_4$) or Mrp2 expression. In contrast, Johnson et al. (2002) recently reported that phenobarbital treatment of rats had no effect on Mrp2 mRNA expression but increased Mrp2 protein expression. As indicated above, treatment of rats with PCN has no significant effect on Mrp2 mRNA expression but increases Mrp2 protein expression several fold (Johnson et al., 2002). We have also observed that PCN treatment of rats in vivo has no significant effect on Mrp2 mRNA but markedly (2- to 3-fold) increases Mrp2 protein expression (Vore et al., 2001). Clearly, further studies are needed to understand the factors regulating Mrp2 expression in vitro versus in vivo. The fact that Mrp2 expression and activity is regulated at three levels, resulting in altered expression within minutes (endocytic retrieval, exocytic insertion), several hours (translational regulation), or days (transcriptional regulation) suggests that control of Mrp2 activity is critical for cell function. The likely role of Mrp2 in efflux of oxidized and reduced glutathione and regulation of intracellular redox status may be such a critical cellular function.

**Transport and ATPase Activity of Mrp2**

MRP2 is known to transport a wide variety of compounds, including various endobiotics and xenobiotics. The substrate specificities of MRP1 and MRP2 are similar, with both extruding glutathione, glucuronide, and sulfate conjugates (Keppler et al., 1997). MRP1 has a higher affinity for leukotriene C$_4$, but bilirubin mono- and bis-glucuronides are better substrates for MRP2 (Keppler et al., 1997). The transport efficiency ($V_{\text{max}}/K_m$) of substrates for rat and human MRP2 have been ranked as follows: leukotriene C$_4$ > leukotriene D$_4$ > 2,4DNP-SG > monogluconosyl bilirubin > E$_2$17G > taurolithocholate sulfate > oxidized glutathione (Keppler et al., 1997).

The substrates and inhibitors of Mrp2 have been thoroughly reviewed elsewhere (Konig et al., 1999a; Suzuki and Sugiyama, 1999). Much of this work defining the substrates of Mrp2 has been done by comparing functional activity in the MRP2-deficient GY/TR$^{-}$ or EHB rats to control rats. Briefly, leukotrienes C$_4$, D$_4$, and E$_4$, and numerous glutathione conjugates, including oxidized glutathione, DNP-SG, bromosulphathalein glutathione, conjugates of heavy metals including arsenic, and cadmium are substrates as shown in either in vitro or in vivo studies (Suzuki and Sugiyama, 1999). Glucuronide conjugates of bilirubin, estradiol, triiodothyrone, gremapfoxacin, and SN-38 are also MRP2 substrates (Suzuki and Sugiyama, 1999), as are numerous conjugates of other compounds, such as acetylanophen glucuronide (Xiong et al., 2000). Additionally, MRP2 transports glucuronide and sulfate conjugates of several bile salts, a range of unconjugated organic anions, including bromosulphathailein, reduced folates, methotrexate, irinotecan, and its metabolite SN-38, ampicillin, ceftiraxone, pravastatin, temocaprilat, and BQ-123, as well as Flu-3 and p-aminophenylurate (Konig et al., 1999a; Suzuki and Sugiyama, 1999; Kusuhara and Sugiyama, 2002). Recently, a chemoprotective role for Mrp2 against carcinogens has been shown for the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol and for the food carcinogen PhIP (Dietrich et al., 2001a; Leslie et al., 2001). Also, the fungal toxin ochratoxin A is an MRP2 substrate (Leier et al., 2000), and transport of the tea flavonoid epicatechin was inhibited by MK571, an antagonist of MRP2, suggesting it also may be an MRP2 substrate (Vaidyanathan and Walle, 2001). These and other data show that MRP2 can transport conjugates, unconjugated compounds, or certain agents in association with glutathione.

In addition to the Mrp2 deficient GY/Tr$^{-}$ or EHB rats, transport activities of MRP2 and mechanisms have been examined in several different expression systems, including transfected mammalian cells and baculovirus infected insect cells. When human MRPI and MRP2 are expressed in Sf9 insect cells using the baculovirus expression vector system, MRP2 has a lower affinity for leukotriene C$_4$ and N-ethylmaleimide-glutathione (NEM-SG) than does MRPI; also, methotrexate is a substrate for both transporters, but its transport by MRP2 is more efficient (Bakos et al., 2000). The effects of several organic anions on NEM-SG uptake by MRP1/2 were also examined (Bakos et al., 2000). Notably, sulfipyrazone ($<300 \mu M$) stimulated MRP2-mediated transport but inhibited MRPI transport; benzylpenicillin and indomethacin also stimulated MRP2 under certain conditions; methotrexate and glutathione were low potency inhibitors of MRPI/2 (Bakos et al., 2000).

Aside from transporting covalent glutathione, glucuronide, and sulfate conjugates, MRP2 can transport certain compounds in the presence of glutathione. Thus, when expressed in Sf9 cells, rabbit Mrp2 did not transport vinblastine in the absence of reduced glutathione, whereas in the presence of 5 mM reduced glutathione, there was significant vinblastine uptake (Van Aubel et al., 1999). Furthermore, leukotriene C$_4$ uptake was stimulated by reduced glutathione and inhibited by vinblastine in the presence, but not absence, of reduced glutathione. Additionally, uptake of reduced glutathione alone was not ATP-dependent, unlike the uptake of conjugates or cotransport of vinblastine and reduced glutathione (Van Aubel et al., 1999).

In a separate study, Evers et al. (2000) further examined vinblastine and sulfipyrazone efflux associated with glutathione in confluent MDCKI cells expressing MRPI or MRP2. Apical efflux of reduced glutathione was inhibited by benzamorene and probenecid but stimulated by low concentrations of sulfipyrazone and indomethacin (Evers et al., 2000). The transport ratio of sulfipyrazone (0.2 to 3.2 mM) to reduced glutathione ranged from 3.1 to 91, suggesting that at low concentrations sulfipyrazone transport is coupled to reduced glutathione but at high concentrations is transported without reduced glutathione (Evers et al., 2000). Furthermore, reduced glutathione apical export increased with increasing vinblastine concentrations, and the transport ratio of vinblastine to reduced glutathione ranged from 2 to 3 (Evers et al., 2000). Unlike MRP2, MRPI transports daunorubicin (Bakos et al., 1998), but this transport is not stimulated by reduced glutathione (Evers et al., 2000). MRP2-mediated reduced glutathione-coupled transport has recently been shown for other toxic compounds as well, including arsenite and $\alpha$-naphthylsulthioxyacetate (Kala et al., 2000; Dietrich et al., 2001b). These two compounds form reversible complexes with reduced glutathione, which dissociate in bile so that the parent compound recycles back to hepatocytes (Dietrich et al., 2001b). This recycling can lead to depletion of intracellular reduced glutathione and also leads to very high
concentrations of α-naphthylisothiocyanate in the biliary tree, its primary site of toxicity.

Excretion into bile involves several steps, including uptake into the hepatocyte, metabolism and transport across the canalicular membrane. Therefore, compounds excreted from the blood into bile may require protein-mediated transport across both the sinusoidal and canalicular membranes. To investigate the functional interplay between uptake and excretion processes, the basolateral transporter human organic anion transporting polypeptide OATP8 (SLC21A8) and the apical transporter MRP2 were coexpressed in MDCKII cells (Cui et al., 2001). When the cells were grown to confluent monolayers, transcellular transport of bromosulfophthalein, anion transporting polypeptide OATP8 (SLC21A8) and the bile acid transporter MRP2 were coexpressed in MDCKII cells (Cui et al., 2001). Similarly, Sasaki et al. (2002) coexpressed Fluo-3, and rifampin was higher in double-transfected monolayers, transcellular transport of bromosulfophthalein, anion transporting polypeptide OATP8 (SLC21A8) and the bile acid transporter MRP2 were coexpressed in MDCKII cells (Cui et al., 2001). When the cells were grown to confluent monolayers, transcellular transport of bromosulfophthalein, anion transporting polypeptide OATP8 (SLC21A8) and the bile acid transporter MRP2 were coexpressed in MDCKII cells (Cui et al., 2001). When the cells were grown to confluent monolayers, transcellular transport of bromosulfophthalein, anion transporting polypeptide OATP8 (SLC21A8) and the bile acid transporter MRP2 were coexpressed in MDCKII cells (Cui et al., 2001).

Regulation of expression
Renal failure
Pregnancy and lactation
Cholestasis
MRP2 mutations
Sepsis (lipopoly saccharide)
Hypo- or hyperosmolarity
Phalloidin
Bile duct ligation
Acute-phase response
Rifampin
FXR, CAR, and FXR ligands
Dibutyryl cAMP
Inhibition or stimulation of activity
ATP depletion
Estrogen glucuronides
Cholestasis
MRP2 mutation: R1150H Cancer chemotherapeutics: irinotecan, methotrexate, and vinblastine
Uricosurics: sulfipyrazone, probenecid, and benzbromarone

Table 2

Factors altering MRP2/Mrp2 mRNA or protein expression and function in vitro or in vivo, as discussed in the text

| Regulation of expression | Renal failure | Pregnancy and lactation | Cholestasis | MRP2 mutations | Sepsis (lipopoly saccharide) | Hypo- or hyperosmolarity | Phalloidin | Bile duct ligation | Acute-phase response | Rifampin | FXR, CAR, and FXR ligands | Dibutyryl cAMP | Inhibition or stimulation of activity | ATP depletion | Estrogen glucuronides | Cholestasis | MRP2 mutation: R1150H | Cancer chemotherapeutics: irinotecan, methotrexate, and vinblastine | Uricosurics: sulfipyrazone, probenecid, and benzbromarone |
|-------------------------|--------------|-------------------------|------------|----------------|----------------------------|------------------------|-----------|-------------------|----------------------|----------|-------------------------|----------------|--------------------------------|--------------|----------------------|-------------|-------------------------|------------------------------------------------------------------|

In summary, alterations in MRP2 expression and/or function could have a variety of clinically important effects. First, decreased MRP2 function can impair normal hepatic function including the capacity to excrete endogenous compounds, such as conjugates of bilirubin, steroids, and leukotrienes. For example, Dubin–Johnson syndrome patients lacking functional MRP2 have hyperbilirubinemia and dark pigment deposition in the liver. Next, altered MRP2 function can change the clearance of many clinically important drugs, including cancer chemotherapeutics (irinotecan, methotrexate, and vinblastine), antibiotics (ampicillin, ceftriaxone, and rifampin), antihyperlipidemics, and angiотensin-converting enzyme inhibitors, as well as many toxins and their conjugates, such as α-naphthylisothiocyanate, heavy metals, 4-(methyl)nitrosamino)-1-(3-pyridyl)-1-butanol, and some dietary compounds, such as ochratoxin A, epicathechin, and PhIP. Since MRP2 seems to act as a protective barrier in the
brain, intestine, and placenta, MRP2 alterations may also affect the absorption and distribution of these compounds, thus affecting therapeutics or toxicology. MRP2 also has a role in drug- and estrogen-induced cholestasis, although the exact mechanism is unclear. Finally, since both reduced and oxidized glutathione are MRP2 substrates, stimulation or inhibition of MRP2 expression or activity may play an important role in cell redox status or response to oxidative stress. Future studies are needed to clarify these complex interactions and the molecular details of MRP2 regulation, structure, and function. Also, further studies are needed to reveal any polymorphisms and their effect on MRP2 function.

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References


