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Mini-review

# MRP2 and 3 in health and disease

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**Abstract**

MRPs are membrane proteins transporting organic anions at the expense of ATP hydrolysis. MRP2 is known to be a major transporter of organic anions from the liver into bile. We discuss recent results showing allosteric control of human but not rat MRP2. MRP3 has been considered a major player in bile salt metabolism, but our recent results with Mrp3 KO mice do not support this. Instead, we have found a role for MRP3 in the cellular export of drug–glucuronide conjugates. We discuss problems in extrapolating results obtained for murine MRPs.


*Keywords:* Multidrug-resistance; Transport; Bile acids; Morphine; Allosteric stimulation

## 1. Multidrug resistance (associated) proteins (MRPs)

Following the identification of multidrug-resistant (MDR) cancer cell lines that did not contain elevated P-glycoprotein (Pgp) levels, Susan Cole and Roger Deeley and collaborators discovered MRP1, responsible for the MDR phenotype of some of these 'non-Pgp' variants. MRP1 proved to be a large membrane protein, present both in the plasma membrane and in an endosomal compartment. Its putative membrane topology is shown in Fig. 1. Besides a core structure of 12 transmembrane segments and two ATP-binding domains, MRP1 has an additional N-terminal domain that is not essential for catalytic function.

Although overexpression of *MRP1* can confer a MDR phenotype on cells that resembles resistance produced by *Pgp* overexpression, the substrate preference of MRP1 proved broader than that of Pgp. In fact, MRP1 is an organic anion transporter that can also transport neutral or weakly basic organic compounds in the presence of GSH or a GSH analog. Among the organic anions transported by MRP1 is also LTC4, and MRP1 has been shown to be the high-affinity transporter of LTC4 in the body.

Since 1992, eight additional human MRPs have been found and a directed search of the human genome has only yielded one additional pseudo-gene, suggesting that the human MRP family is now complete. The MRPs have all been placed in the ABCC subfamily of ATP-binding cassette transporters and Table 1 summarizes a few key features of the MRPs characterized thus far. In this review we focus on MRP2 and MRP3. The older work on these transporters can be found in recent reviews [1–4]. Here we discuss issues that have come up recently.

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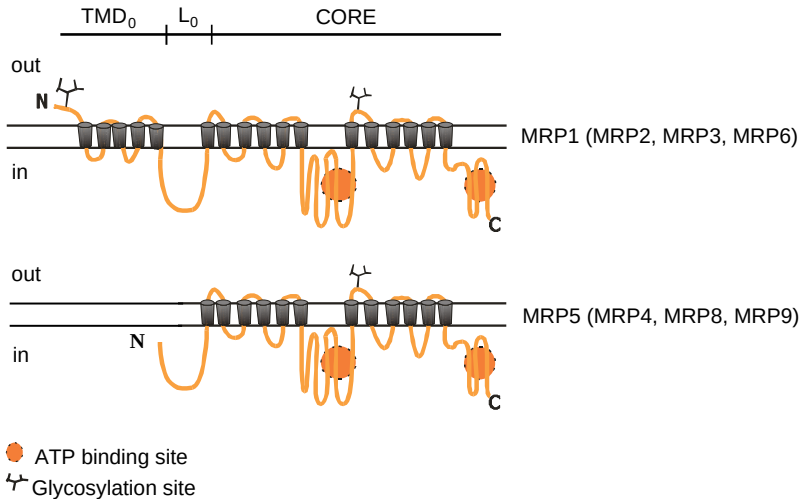


Fig. 1. Models depicting the putative membrane topology of MRP1 and MRP5. MRP2, MRP3 and MRP6 have a structure similar to MRP1 and are characterized by the presence of the extra N-terminal extension with five transmembrane regions ($TMD_0$) connected to the Pgp like core by a cytoplasmic linker $L_0$. MRP4, MRP8 and MRP9 resemble MRP5.

## 2. Techniques

Four methods have been mainly used to identify the substrates transported by MRPs and to identify the main functions of these proteins:

1 Analysis of cells transfected with constructs allowing overexpression of an MRP. This approach has mainly been used to study drug resistance and to determine the form of the drug transported out of the cell by the MRP. As a counterpart of this approach, one can also knock down the expression of an MRP gene in cells in which it is endogenously expressed by RNAi and verify the effects of these knock-downs on drug transport and drug resistance. The paper by Reid et al. [5] gives an example, showing how knock-down of the low level of MRP4 expression in HEK293 cells results in a strong

Table 1
Several general features of MRPs

| | Main localization in the body | Drug substrates | Physiological substrates | Proposed function | Phenotype of KO mice |
|---|---|---|---|---|---|
| MRP1 | Ubiquitous | Anthracyclines, Vinca alkaloids, Epipodophyllotoxins, Campyothecins, Methotrexate | LTC4, GSH | Modulation of immune response, protection of tissue against xenobiotics | Drug hypersensitivity |
| MRP2 | Liver, kidney, gut | Anthracyclines, Vinca alkaloids, Epipodophyllotoxins, Campyothecins, Methotrexate, Cisplatin | Bilirubin glucuronide, $E_2$17βG, GSSG, acidic bile salts | Biliary excretion of organic anions, bile salt independent bile flow, protection against xenobiotics | Altered drug handling/ disposition |
| MRP3 | Liver, kidney, gut, pancreas, lung, adrenals | Epipodophyllotoxins, Methotrexate, drug–glucuronides, drug-conjugates | Bilirubin glucuronide, $E_2$17βG, (glucuronidated) bile salts | Protection against xenobiotics | Altered drug handling/ disposition |
| MRP4 | Prostate, lung, muscle, pancrea, testis, ovary, bladder, gallbladder | Nucleotide analogs, Methotrexate | Cyclic nucleotides, prostaglandins | ? | ? |
| MRP5 | Ubiquitous | Nucleotide analogs, Methotrexate | Cyclic nucleotides | ? | ? |
| MRP6 | Liver, kidney | | BQ-123 | Elastic tissue homeostasis | No KO available |
| MRP8 | ? | Nucleotide analogs | Cyclic nucleotides | ? | No KO available |
| MRP9 | Testis, brain | | | ? | No KO available |

decrease in the MRP4-mediated export of prostaglandin E from the cell.

2 Analysis of MRP-mediated drug transport in monolayers of polarized cells transfected by a gene construct allowing expression of the MRP gene (Fig. 2). Depending on the MRP, the transporter will either be routed to the apical or basolateral membrane (see Fig. 2(A)), allowing vectorial transport of the MRP substrates. As the substrates of MRPs are usually organic anions, which do not penetrate the plasma membrane easily to enter the cell, this approach has been less useful for MRPs than for Pgp. Attempts have been made, however, to overcome this limitation by introducing a second transporter construct into the monolayer cells to allow import of the organic anions to be transported by ABC transporters [6–9].

3 Vesicular transport experiments. The system used most extensively are insect Sf9 cells infected with a baculovirus construct allowing very high expression of the transporter of interest. However, also transfected mammalian cells, such as HEK 293 and LLC-PK1 cells, have been used for this purpose. In the inside–out plasma membrane vesicles the transporter is directly in contact with the drug in the medium and the system is therefore suitable for determining the affinity of the substrate for the transporter ($K_m$) and the transport rate ($V_{max}$). The method is especially useful for MRPs, because most of the substrates of these transporters are negatively charged and poorly membrane permeable. Hence, the substrate transported into the vesicles does not leak out rapidly, although there are exceptions. For instance, prostaglandins E1 and E2 leak out at substantial rate [5].

4 KO mice in which both alleles of the transporter gene have been disrupted by stem cell technology. In some cases natural null mutants are known in rodents or humans that have provided important information about transport function. In several cases, however, the KO mice have no apparent defect (Table 1) and in these cases the physiological function of the transporter is still in doubt.

## 3. Multidrug resistance protein 2 (ABCC2); general features

Although MRP2 is the second MRP in name, it was actually the first MRP discovered and characterized. Already in 1985, Jansen and co-workers identified a mutant rat strain, the TR$^-$/GY rat, with a defect in the excretion of bilirubin–glucuronides and other organic anions into bile [10]. A similar rat mutant, the Eisai hyperbilirubinemic rat (EHBR), was identified in Japan. We now know that these rat mutants are defective in MRP2, like humans suffering from the Dubin–Johnson syndrome. Biochemical characterization of MRP2 started already in 1990, when Ishikawa et al. [11] found an ATP-dependent transporter in the canalicular membrane of hepatocytes, which was able to transport glutathione-*S*-conjugates. Subsequent work

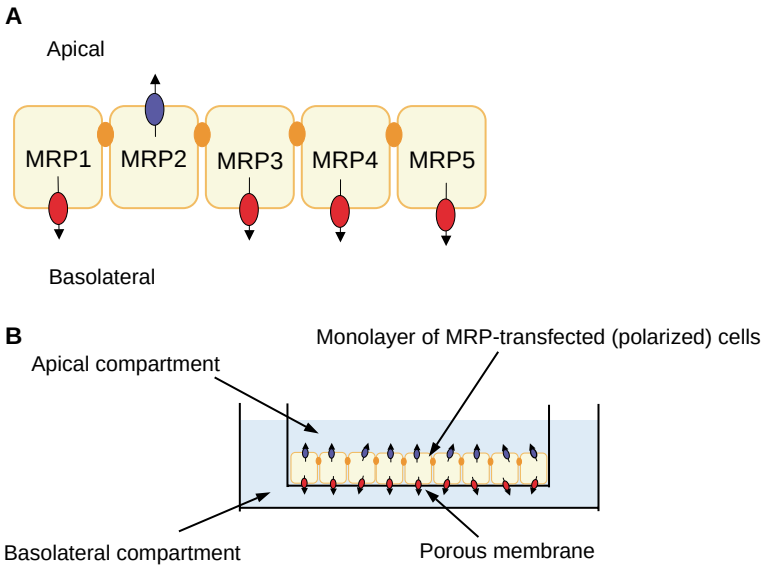


Fig. 2. (A) Schematic representation of the localization of the MRPs in (transfected) polarized epithelial cells and (B) experimental setup of a transwell experiment using polarized epithelial cells transfected with a MRP cDNA construct.

by Oude Elferink et al. [12] showed that this transporter was absent in the $TR^-$/GY rats and that it transported several organic anions. Hence, the name canalicular multispecific organic anion transporter (cMOAT). When the cloning of the human and rat *MRP2* genes showed in 1996 that MRP2 is a close relative of MRP1, the (rather appropriate) cMOAT name was replaced by MRP2 [13].

Although high overexpression of *MRP2* renders cells resistant to several anti-cancer drugs, resistance is modest and there is no correlation between MRP2 level and clinical resistance in cancer patients [14]. In contrast, the essential role of MRP2 in transporting organic anions is not in doubt. Strategically located at the apical membranes of hepatocytes and renal tubular cells, it contributes to the excretion of normal metabolites and drug (-conjugates). The location of MRP2 in the intestinal mucosa allows it to limit uptake of some drugs and other xenotoxins into the body via the oral route.

Comprehensive reviews present a detailed overview of the older literature [2,4,13,15]. Here, we analyze recent data on MRP2 modulation.

## 4. Modulation of the activity of multidrug resistance protein 2

Work in several labs has established the presence of multiple drug binding sites within MDR1 Pgp, an MRP2-related ABC multidrug transporter. These sites differ in their drug-binding properties and in their ability to promote transport of a bound drug. Interestingly, these sites do not behave independently, but display complex allosteric interactions, e.g. binding of a drug to one site may increase binding and transport of a drug from a different site [16,17].

Not surprisingly, indications that MRPs contain multiple binding sites have turned up as well. Work from the lab of Cole and Deeley demonstrated that GSH and vincristine can reciprocally stimulate their transport by MRP1 in isolated vesicle membranes [18]. Subsequently, several groups showed that transport by other members of the MRP family can also be modulated by a diverse array of compounds [6,19–21] . Evers et al. [22] found that in polarized MDCK/MRP2 cells sulfinpyrazone stimulates the apical secretion of GSH leading them to propose that MRP2 contains separate transport sites for drugs and for GSH (the D and G sites, respectively) from which drugs and GSH are co-transported by MRP2.

Recent results, however, do not fit this model [20,23,24]. Several compounds that stimulate transport of substrates by MRP2 are themselves not detectably transported [20,23]. The main MRP2 substrate used in these studies was estradiol-17β-glucuronide ($E_2$17βG), a cholestatic estradiol metabolite. Unexpectedly, we and others found that this substrate is transported by human [23,24], rat [25,26], and dog [26] MRP2/Mrp2 with complex kinetics that did not follow simple Michaelis–Menten kinetics, as shown in Fig. 3, panel A. The transport rate vs. substrate concentration gives a sigmoidal curve indicative of (minimally) two $E_2$17βG binding sites that display positive cooperativity. Whereas the results for human MRP2, obtained in two different labs are similar [23,24], the results for rat Mrp2 are not, as illustrated in panels B–D of Fig. 3 and in Table 2. We find a curve that is compatible with Michaelis–Menten kinetics (Fig. 3(B)) and the transport is only minimally stimulated by compounds such as sulfanitran and indomethacin, that stimulate transport by human MRP2 more than 10 fold [23]. In contrast, studies by Gerk et al. [25] and Ninomiya et al. [26], suggest that also rat Mrp2 has two $E_2$17βG binding sites.

The large differences obtained with $E_2$17βG as substrate in different labs are remarkable and most likely not due to trivial differences in experimental set up. The results presented in Fig. 3(B)–(D) were obtained in three different labs that all used the Sf9/baculovirus expression system. Moreover, two labs used the same rat cDNA construct (Fig. 3(B) and (C)), whereas the rat Mrp2 studied by the third lab differs only at position 420 (M:V). It is difficult to see how one could get reliable $K_m$ and $V_{max}$ values if the results in different labs are so different. An explanation worth exploring is that the exact composition of the membrane is critical to the results obtained. MRP2 functions within a membrane and probably takes its substrate from this phase. The substrates are amphipathic and partition to various degrees into the membrane. The exact membrane composition could therefore be critical to the transport properties of the transporter embedded in it, and it is conceivable that these membrane properties are affected by the exact growth conditions used for the Sf9 cells. In the same fashion modulators might not only affect the transporter protein, but also the membrane composition in the environment of the transporter [27]. A systematic test of these variables would be useful. Given these uncertainties, some caution should be exercised in extrapolating results obtained in vesicular transport experiments using Sf9 vesicles to the mammalian cells in which these transporters normally function.

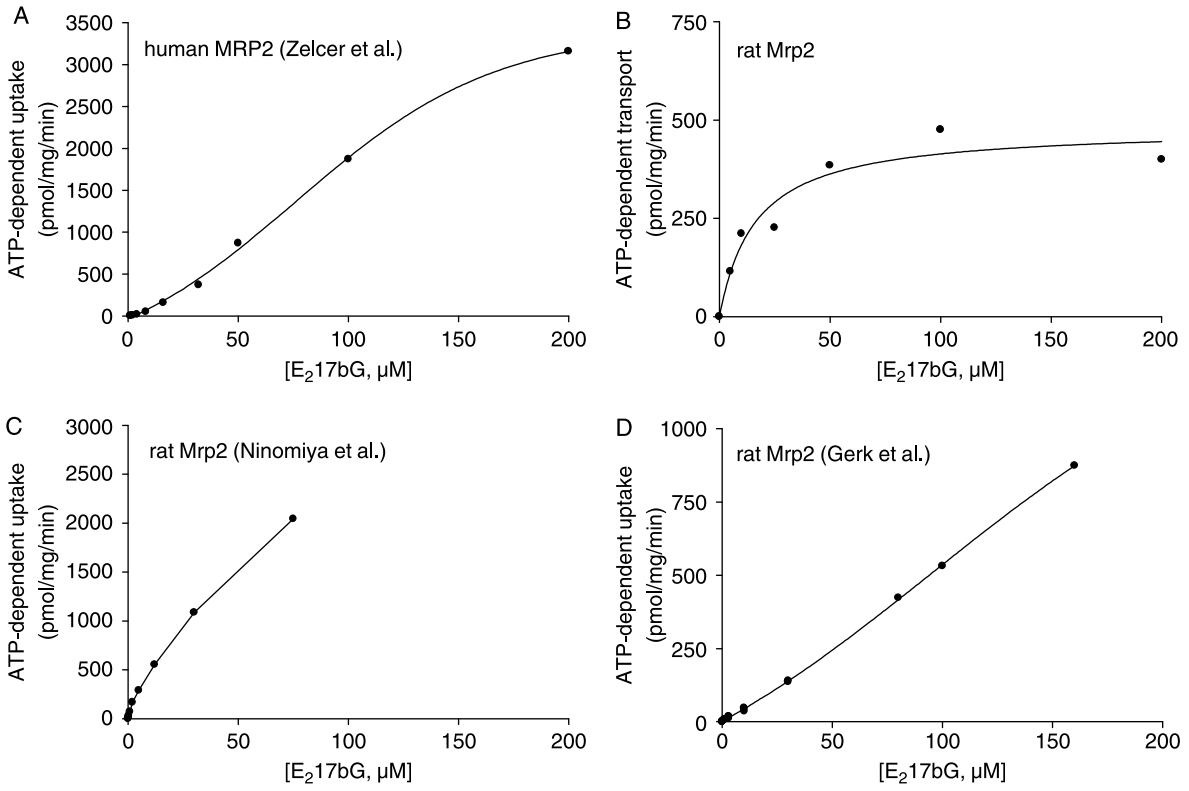


Fig. 3. Concentration dependent transport of $E_2$17βG by human and rat Mrp2: (A) transport by human MRP2 [23]; (B, C, D) transport of $E_2$17βG by rat Mrp2 as determined in different labs. Data for panels C and D were kindly provided by Gerk et al. [25] and Ninomiya et al. [26], respectively. Panel B represents $E_2$17βG transport by rat Mrp2 as determined in our lab.

Notwithstanding the variation in results obtained in different labs, there is no dispute that MRP2/Mrp2 contains (minimally) two $E_2$17βG binding sites (Fig. 4). Two different models have been proposed to explain the positive cooperativity of MRP2/Mrp2 mediated $E_2$17βG transport:one model (Fig. 4(B)) with a single transport (T) site and a modulatory (M) site [23,25]; and one with two transport sites, which can modulate each other [19,24]. In the single-site model, binding of a compound to the M site results in increased affinity of the T-site for its substrate. The M site is postulated to be unable to mediate transport, as we identified several compounds that strongly stimulate transport of $E_2$17βG but are themselves not detectably transported [23]. Consistent with this model is the finding of Chu et al. [20] that ethinylestradiol-3-sulfate (EE3S) strongly

Table 2
Kinetic parameters for $E_2$17βG transport by MRP2/Mrp2 obtained in different laboratories

| Recombinant transporter | Cells used for preparation vesicles | $K_m$ (μM) | $V_{max}$ (pmol/mg membrane protein/min) | Shape of concentration dependent uptake curve |
|---|---|---|---|---|
| Human MRP2 (Cui) | HEK293 | 7.2±1.8 | 144 | Not reported |
| Human MRP2 (Zelcer) | Sf9 | Not calc.[a] | >3000[b] | Sigmoidal |
| Human MRP2 (Bodo) | Sf9 | Not calc.[a] | >10,000[b] | Sigmoidal |
| Rat Mrp2 (Cui) | HEK293 | 6.9±1.8 | 110 | Not reported |
| Rat Mrp2 (Ito) | Sf9 | 3.9±0.9 | ND | Not reported |
| Rat Mrp2 (Niyomiya) | Sf9 | 4.8±1.2/$PS_{NS}$[c] | 390[b] | Michaelis–Menten |
| Rat Mrp2 (Gerk) | Sf9 | Not calc.[a] | NS[b] | Sigmoidal |
| Rat Mrp2 (our) | Sf9 | 16±6 | 482±48 | Michaelis–Menten |
| Dog Mrp2 | Sf9 | 3.3±0.1 | 60±10 | Sigmoidal |

[a] Transport could not be saturated and no $V_{max}$ and $K_m$ determined.
[b] Computer calculated in Ref. [26] NS, not saturated.
[c] Non-saturable component of transport.

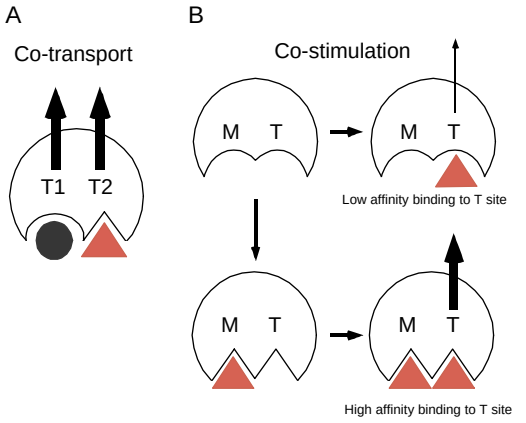


Fig. 4. A schematic representation of the two mechanistic models of MRP-mediated transport. The co-transport model (A) assumes that two substrates that stimulate each other's transport are transported together in a transport cycle via the transport sites T1 and T2. The two-site model (B) assumes the existence of a modulatory site that only modulates transport and a transport site from which substrates are transported [23].

stimulates MRP2-mediated transport of ethinylestradiol-3-glucuronide (EE3G), but is itself not transported. The sigmoidal V vs. [S] curve of MRP2-mediated $E_2$17βG transport is explained by assuming that $E_2$17βG binds with higher affinity to the M site than to the T-site, thereby stimulating its own transport. $E_2$17βG is thus far the only MRP2 substrate found that is able to stimulate its own transport, but this may be due to the fact that many substrates cannot be tested, because they are not available in radioactive form. For instance, the MRP2 substrate sulfinpyrazone [22] stimulates $E_2$17βG transport at low concentrations, but inhibits at higher concentrations, like several other compounds [23]. We interpret this to mean that sulfinylpyrazone binds preferentially to the M-site at low concentrations stimulating $E_2$17βG transport; at high concentrations it also binds to the T-site, competing for transport and therefore lowering the (stimulated) $E_2$17βG transport. Other compounds only inhibit transport of $E_2$17βG by MRP2 (e.g. GS-DNP). According to our model these compounds only interact with the T-site. Gerk et al. [25] explain their data (Fig. 3(D)) along similar lines, but they deduce that the M-site in their rat Mrp2 has a low affinity for $E_2$17βG. From the inhibition of transport by other glucuronide conjugates and by sulfate conjugates of estradiol, they conclude that the T-site is more permissive than the M-site.

Bodo et al. [24] favor a two-site co-transport model for MRP2, mainly based on their observation that $E_2$17βG and glycocholate (GC) stimulate transport of each other and especially the fact that the 'the combined transport rate for $E_2$17βG and GC is significantly greater than that expected from additive effect of these two substrates' [24]. We are not convinced by this cross-stimulation argument. If both substrates bind to the M-site and substantially stimulate transport through the T-site, the stimulation of over-all transport could greatly exceed the competition of both substrates for the T-site. This would also explain the apparent co-transport observed by Evers et al. [22].

The allosteric modulation of MRP2 is not an artefact of vesicular transport experiments, as sulfanitran stimulates the vectorial transport of saquinavir [23] and other antiviral drugs [28] in MDCK-II cells overexpressing MRP2. Moreover, Ito and coworkers [29] recently showed that benzylpenicilin, a strong stimulator of $E_2$17βG [23,29] and GS-DNP [29] transport by MRP2, also stimulated biliary GSH and conjugated bilirubin transport in normal rats but not in EHBR rats which are deficient in Mrp2.

The possible role of these interactions in physiology requires more experiments. The ability of a substrate to stimulate its own transport would allow MRP2 in the liver to respond to a wide range of substrate concentrations without being saturated, as was also suggested for drug-metabolizing enzymes [30]. Transport of $E_2$17βG by MRP2 is stimulated by taurocholate and glycocholate at concentrations found in normal liver [23,24], suggesting that MRP2 can respond to fluctuating levels of these major bile salt species. Chu et al. [20] found that ethinylestradiol-3-sulfate is not transported by MRP2, but able to stimulate transport of ethinylestradiol-3-glucuronide and $E_2$17βG more than 10 fold. Both compounds are made in the body from ethinylestradiol, present in many oral contraceptives. This will be a fruitful area for future pharmacokinetic studies.

## 5. Multidrug resistance protein 3 (ABCC3); general features

MRP3 is the MRP family member most closely related to MRP1 [3,4]. It also routes to the basolateral membrane of epithelial cells, but its tissue distribution is much more restricted than that of MRP1. It is mainly present in liver, adrenals, pancreas, kidney, gut gallbladder, and it transports a variety of organic anions with a preference for glucuronosyl- and sulfate conjugates. Several polar residues in transmembrane domain 17 are involved in the substrate specificity of MRP3 [31]. Interestingly, elimination of the hydrogen bonding potential of a single amino acid in

transmembrane domain 17 ($Thr^{1237}$) enhanced the ability of the protein to confer drug resistance and to transport all substrates that were examined in that study [31].

Overexpression of *MRP3* results in resistance to etoposide/teniposide and MTX in short-term/high-concentration regimens, but not to any other anti-cancer drug tested. In fact, MRP3 is not really a multidrug resistance-associated protein in the literal sense of the word. The fact that an association has been found between poor response to chemotherapy and increased levels of MRP3 (mRNA) in several tumors [32–34] does not contradict this conclusion. We know many examples of apparent correlations between resistance and resistance markers, where the resistance protein was found not to contribute to the resistance observed. A nice example is the major vault protein [35]. In the case of MRP3 clinical associations have been reported for drugs not transported at sufficient rate by MRP3 to cause resistance in transfected cells [36]. Hence, we think that claims for the clinical significance of MRP3 in MDR are not persuasive.

The spectacular modulation of MRP2 transport by compounds like sulfinpyrazone or indomethacin (Section 4), has not been reported for MRP3. We have observed up to threefold stimulation of the transport of 1 μM $E_2$17βG by methylumbelliferone sulfate, but not by the glucuronide derivative [6], similar to what was observed with rat Mrp3 [37]. Bodo et al. [38] reported a similar modest stimulation by benzbromarone. Stimulation of MRP3-mediated transport is not limited to $E_2$17βG, as Chu et al. [20] found that transport of the glucuronide derivate of ethinylestradiol (EE-G) could be stimulated by the sulfate conjugate of the same compound (EE-S) up to fivefold. No stimulation by metabolites normally present in tissues containing MRP3 has been reported, but no systematic search for such stimulations has been done.

## 6. Does MRP3 have a physiological role in bile salt transport?

At an early stage, Sugiyama's group discovered that the cloned rat Mrp3 transported bile salts rather effectively. Taurocholate was transported with a $K_m$ of 16 μM and a $V_{max}$ of 50 pmoles/min/mg protein; taurolithocholate-3-sulfate with a $K_m$ of 3 μM and a $V_{max}$ of 162 pmoles/min/mg protein [39]. In other labs human MRP3 was found to transport bile salts as well, but with much lower affinity [6,36,40]. Akita et al. [41] compared rMrp3 with hMRP3 under identical conditions in Sf9 insect-cell vesicles and confirmed the much lower affinity of the human transporter for taurocholate and glycocholate. Akita et al. [42] also showed an enhanced efflux of taurocholate from the liver into the blood in Mrp2-deficient rats. The rate of efflux correlated with the level of Mrp3 in the sinusoidal liver membrane, 'suggesting a contribution by Mrp3 to the sinusoidal efflux of taurocholate' [43].

The generation of a Mrp3 KO mouse made it possible to test this suggestion in mice [44,45]. Unexpectedly, there was no difference in serum bile salt levels between wild-type and KO mice after bile duct ligation (BDL). There was no difference in apparent liver damage either. This suggests that in mice Mrp3 does not contribute significantly to disposal of major bile salts from the liver if the efflux into bile is blocked [44,45]. The gut is another tissue where MRP3 was thought to contribute to bile salt transport. Bile salts are taken up by enterocytes via the apical bile salt transporter. They have to get out at the basolateral side and Mrp3, located there, seemed a good candidate for this job. However, absence of Mrp3 had no detectable effect on trans-ileal taurocholate transport [44].

The conclusion from the experiments with the KO mice is therefore that the murine Mrp3 seems unimportant for bile salt transport in vivo. Mice are no rats or humans, however, and this conclusion should not be generalized without further research. It should also be pointed out that Zelcer et al. [44] found glucuronide conjugates of hyocholate and hyodeoxycholate (HDC) to be excellent substrates of MRP3. These conjugates are made in humans, but not in mice. In an attempt to test Mrp3 involvement in the secretion of these compounds from the liver, Zelcer et al. perfused mouse livers with HDC, a substrate they normally do not see in substantial amounts. The mouse liver converted some of this HDC into glucuronosyl-HDC and the sinusoidal secretion of this compound was strongly diminished in the Mrp3 KO mouse [44]. This suggests that MRP3 could contribute to the excretion of these minor glucuronidated bile salts in humans, whose levels increase in some cholestatic states.

## 7. MRP3 contributes to the disposal of drug–glucuronide conjugates

Although the Mrp3 KO mice lack detectable alterations in bile salt handling, they did show one clear difference with their wild-type counterparts: after bile duct ligation their plasma bilirubin–glucuronide level was only half the level in WT mice, suggesting that the sinusoidal excretion of this substrate is in part mediated by Mrp3 [44,45]. This result is in line with

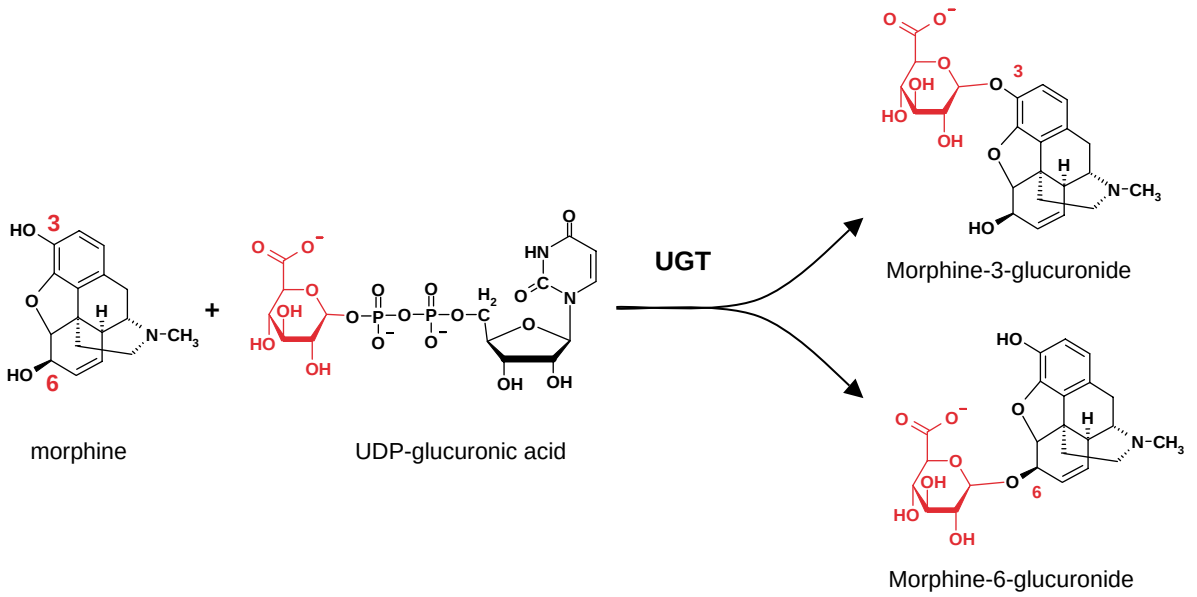


Fig. 5. Schematic representation of the enzymatic conjugation of morphine to glucuronic acid. Conjugation of morphine to glucuronic acid mainly occurs in the liver and in humans is catalyzed by the UGT2B7.

the data from vesicular transport experiments, which suggest that Mrp3/MRP3 is particularly good at transporting glucuronosyl conjugates. To test whether basolateral Mrp3 is an important part of the hepatic detoxification pathway in which drugs are conjugated to glucuronate, transported out of the cell and excreted in the urine, we have used morphine as a model. Morphine is rapidly taken up by the liver and converted into glucuronosyl–morphine by one of the UDP-glucuronosyl transferases present (Fig. 5). In mouse liver only morphine-3-glucuronide (M3G) is formed; humans also make morphine-6-glucuronide (M6G). The M3G is in part excreted into bile, in part into the circulation. We have tested whether MRPs are involved in this excretion.

Indeed, we found that both MRP3 [46] and MRP2 (unpublished experiments) transport M3G at high rate, albeit with modest affinity. Nevertheless, this modest affinity is sufficient for the rapid excretion of M3G from the liver, as excretion was lost in the KO mouse, resulting in a 50 fold reduction in M3G plasma levels [46]. It follows that Mrp3 is the only basolateral transporter in the liver able to excrete M3G into the blood. In unpublished experiments in collaboration with A.H. Schinkel we have verified that transport of M3G into bile is exclusively mediated by Mrp2.

These results emphasize the crucial role of MRPs in the disposal of drug–glucuronide conjugates. As schematically indicated in Fig. 6, Mrp2 and Mrp3 normally compete for the M3G formed in the liver. Removal of Mrp3 leads to a complete inhibition of the excretion into blood. The M3G concentration in liver goes up about fourfold and this is sufficient to excrete all M3G into bile [46], because the relatively low affinity of Mrp2 for M3G results in an excess transport capacity that can be recruited for biliary excretion of M3G. We expect that a similar scenario will unfold in the Mrp2 KO mouse, but with all M3G ending up in the blood. This remains to be verified.

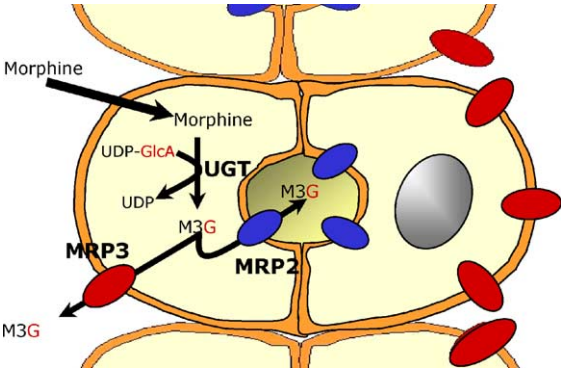


Fig. 6. Schematic representation of the transporters involved in the excretion of morphine-3-glucuronide from the murine liver. Morphine that is taken up from the circulation by hepatocytes is converted into M3G, which can be transported into bile by Mrp2 or back to the circulation by Mrp3. Note that in mice a different UGT is involved in the glucuronidation of morphine, resulting in the formation of morphine-3-glucuronide only.

Given the fact that the affinity of MRP2 for $E_2$17βG can be strongly increased by compounds normally present in the liver (section 4), it is risky to speculate about the actual affinity of Mrp2 and 3 for M3G in their natural environment in mouse liver. It could be much higher than the $K_m > 100$ μM, found in vesicular transport experiments, indicates. Nevertheless, even a low-affinity, high-capacity pump could make sense for drug disposal. It would allow the liver to deal with a large range of drug–glucuronide conjugates and prevent build up of excessive concentrations. The availability of two exits for these compounds, canalicular (Mrp2) and sinusoidal (Mrp3), provides also a high degree of flexibility in case one of the exits gets clogged by other substrates.

We expect MRP3 to play an important role in drug disposal in all tissues where glucuronidation is a prominent drug detoxification mechanism, i.e. liver and gut. That Mrp3 is important in the liver for acetaminophen/paracetamol disposal was recently shown [47]. We are investigating the role of Mrp3 in the export of glucuronide conjugates out of the gut.

## 8. Concluding remarks

Mammals produce a vast amount of organic anions that need to go in and out of cells. Whereas pathways for producing these anions were largely delineated in the second half of the 20th century, the question how they pass the plasma membrane and intracellular membranes was hardly raised. The past 10 years has seen a major research effort to fill this gap. Several large families of organic anion transporters now provide a range of transport options [48–50] and it will require a major research effort to determine the physiological function of each of these transporters. We expect that KO mice will play a key role in this effort.

The function of MRP2 in liver and gut is now fairly well defined; more work is required to determine its role in the kidney and in the blood–brain barrier, however. The function of MRP3 is only slowly emerging. It has an indispensable role in the disposition of drug–glucuronide conjugates and this explains its location in liver and gut, tissues active in drug glucuronidation. Why there should be a high MRP3 level in the adrenal cortex is still unclear, however. Transport of steroid–glucuronides and -sulfates is an obvious possibility, as these are substrates for MRP3. More work is required to determine whether any steroid metabolite accumulates in the adrenals of KO mice.

Defining the physiological function of MRPs will continue to be complicated by the overlapping substrate preferences of MRPs and by the differences between homologous MRPs from humans and rodents. The first example of this was the inability of murine Mrp1 to confer resistance to anthracyclines, like the human MRP1 can. This was traced to a single aminoacid replacement by Deeley and coworkers [51] Since that time many more have been found, as illustrated by the case of MRP2 discussed here. Even if the ongoing generation of double and triple KO mice helps to give a clear picture of Mrp function in mice, it will therefore remain a major task to translate the mouse results to humans.

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