Comparative Analysis of Human Multidrug Resistance-Associated Protein 2 (MRP2) and Rat Mrp2

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Introduction

The human MRP2 transporter (acc. no. Q92887) shows 72.3% sequence identity and 85.6% sequence similaritywith its rat ortolog (acc. no. Q63120). Both transporters are expressed on the canalicular membrane of the liver and are known to be responsible for the transport of some organic molecules and their conjugates to the bile (reviewed in König et al., 1999; Suzuki and Sugiyama, 1998; Chandra P and Brouwer KL, 2004). Naturally occurring mutations resulting in deficiencies of MRP2 and rat Mrp2 function have been described (Kartenbeck et al., 1996; Büchler et al., 1996). These mutations cause increased blood level of conjugated billirubin metabolites and other metabolites in both cases. The human disorder is called Dubin-Johnson syndr (Dubin and Johnson, 1954), while rats deficient in Mrp2 function are called TR- rats (Jansen et al., 1985; Kitamura et al., 1990; Paulusma et al., 1996) or Eisai hyperbillirubinemic rats (Büchler et al., 1996). These mutant strains are widely used as an animal model of human MRP2 function.

Besides conjugates of normal liver function the MRP2 transporter is also known to be responsible for the transport of therapeutics and their conjugates (Oude Elferink et al., 1995; Cui et al., 1999; Xiong et al., 2000). Therefore, it is very important to understand the interaction of drugs with this transporter as it might be crucial for their ADME properties and the overall success of the therapy. Also, it has been reported that the MRP2 transporter has multiple binding sites that is important in the regulation of the transport of some compounds (Zelcer et al., 2003; Bakos et al., 2000).

In the recent years new in vitro methods have been invented to assay the interaction of test drugs with ABC transporters. These methods are available in high throughput format allowing the screening of large number of compounds in a well-defined system. In this study we used the ATPase assay and the vesicular transport assay to compare the substrate specificities and kinetic properties of the human MRP2 and rat Mrp2 transporters. We were interested if the differences in amino acid sequence between the two transporters result in any difference in their biochemical parameters and the regulation of the transporter. As in ADME studies rats are the most commonly used animal models, this information can be very important to see if these animal models yield data that can be directly applied to the humans, or not.

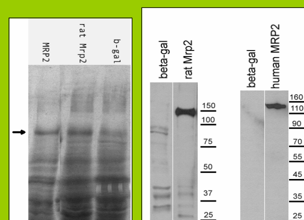

Figure 1. Expression of recombinant MRP2 and rat Mrp2 in Sf9 cells. (A) Membrane vesicles (25 ug/lane) were loaded to 7.5% SDS-PAGE poliacrylamide gel and Coomassie stained after separation. Lanes containing membranes purified from Sf9 cells expressing MRP2 (lane 1) or rat Mrp2 (lane 2) show bands with similar intensities at around 150 kDa (arrow) as expected. This band is much weaker in the lane containing control membranes (lane 3). (B) The presence of MRP2 or ratMrp2 in the membrane preparations as detected by specific monoclonal antibodies using Western blot. 1 μg of membrane protein was loaded to 7.5 % SDS-PAGE and blotted on PVDF membranes. No band in control membrane

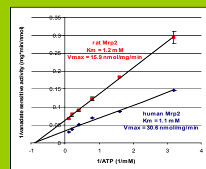

Figure 2. Kinetic constants of the vanadate sensitive ATPase activity of MRP2 and rat Mrp2 membrane vesicles in the presence of a 1 mM and 4 mM Probenecid, respectively. Membranes containing 20 μg of total protein were incubated with 1 mM (human MRP2) or 4 mM (rat Mrp2) probenecid at 37 °C for 60 min in the presence of different concentrations of ATP. Probenecid-stimulated vanadate sensitive ATPase activity was determined.

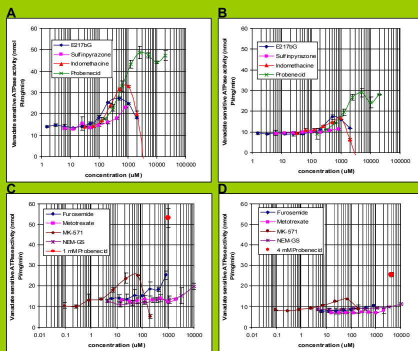

Figure 3. Vanadate sensitive ATPase activity of MRP2 and rat Mrp2 membrane vesicles in the presence of different compounds. MRP2 (A and C) or Mrp2 (B and D) expressing membranes (20 μg of total protein) were incubated at 37°C with drugs at concentrations indicated. After 60 min drug stimulated vanadate sensitive ATPase activities were determined.

	EC$_{50}$ (μM)	
	human MRP2	rat Mrp2
$E_2 17\beta G$	145 +/- 35	385 +/- 49
Indomethacine	240 +/- 57	335 +/- 120
Probenecid	670 +/- 42	1500 +/- 707
MK-571	6.5 +/- 1	30 +/- 7
NEM-GS	4500 +/- 707	No activation
Sulfinpyrazone	440 +/- 14	No activation
Sulfasalazine	27.5 +/- 11	>100
Furosemide	162.5 +/- 53	No activation
Methotrexate	No activation	No activation

Table I. EC$_{50}$ values for compounds tested in the ATPase assay for human MRP2 and rat Mrp2.

Figure 4. ATP dependent vesicular transport of ^{3}H-NEM-GS. Membrane fraction containing inside out membrane vesicles were incubated in the presence or absence of 4 mM ATP and 400 μM ^{3}H-NEM-GS at 37 °C for 4 min. The rate of ATP dependent transport was determined.

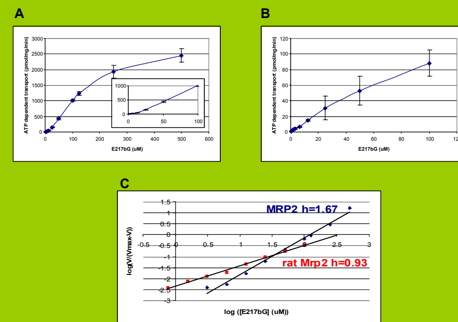

Figure 5. Concentration dependence of ^{3}H-$E_2 17\beta G$ vesicular transport. Membrane fraction containing inside out membrane vesicles were incubated in the presence or absence of 4 mM ATP at 37 °C for 8 minutes for human MRP2 (A), or 2 min for rat Mrp2 (B). ATP dependent transport was determined. Inset depicts transport rate at low ^{3}H-$E_2 17\beta G$ concentrations. (C) Hill plot of curves A and B have been derived.

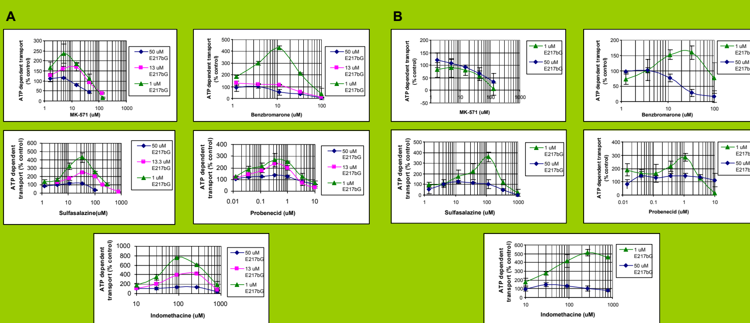

Figure 6. Vesicular transport of ^{3}H-$E_2 17\beta G$ at different $E_2 17\beta G$ concentrations and in the presence of different test drugs for MRP2 (A) and rat Mrp2 (B) vesicles. Membrane fraction containing inside out membrane vesicles were incubated in the presence or absence of 4 mM ATP at the indicated concentrations ^{3}H-$E_2 17\beta G$ and drugs at 37 °C for 16 minutes for human MRP2 (A), or 2 min for rat Mrp2 (B). ATP dependent transport was determined.

Results and disscusion

Using our high throughput ATPase assay system we screened over 100 compounds and found only a few differences between the two transporters with regard to substrate specificity. In this assay system the rat Mrp2 transporter showed significantly higher EC50 values (Table I) than the human ortholog suggesting a somewhat higher Km value for these drugs. In addition, 3 substrates NEM-GS, sulfinpyrazone and furosemide did not activate the rat transporter in the concentration range tested (Table I). Differences in substrate specificities between species is not uncommon in the transporter field (Dresser et al., 2000; Ullrich et al., 1991; Groves et al., 1994).

Vesicular transport studies confirmed the difference in substrate specificity in NEM-GS transport (Fig 4). This difference is somewhat unexpected since rat Mrp2 has been shown to transport DNP-SG (Ito et al., 2001; Ito et al., 1998) a small glutathione conjugate, similar to NEM-GS. In addition, vesicular transport studies have shown that there seems to be a marked difference in the kinetics of the transport of $E_2 17bG$, an important endogenous substrate of MRP2. In correlation with the ATPase data $E_2 17\beta G$ is a higher affinity substrate of human MRP2 than the rat protein. The sigmoid transport curve and Hill plot data have shown that the human protein likely has two binding sites ($n = 1.67$) while the rat protein displays only one site ($n = 0.93$). This is in good agreement with published data showing cooperative transport of $E_2 17\beta G$ for human MRP2 (Zelcer et al., 2003) and a single functional site for the rat Mrp2 (Ninomiya et al., 2004). The phenomenon of cooperativity is not restricted to the human protein since heterotropic effect by many compounds is obvious from our data (Fig. 5) and has been previously shown by others (Ninomya et al., 2004).

There is a clear difference, however, in the specificity profile with MK-571 stimulating transport of $E_2 17\beta G$ only by the human MRP2 (Fig 6). It is also evident that the compounds have lower affinity for the modulatory site on the rat protein than the equivalent site on the human MRP2 (Fig 6). It has a pivotal importance with MK-571 since MK-571 potentiates transport of human MRP2 in the low micromolar range, a range that it is often used at as an MRP inhibitor in monolayer efflux assays.

The potentiating effect of different substrates depend on the concentration of $E_2 17\beta G$. At 50 μM concentration of $E_2 17\beta G$ only inhibition is seen. With the two-site model proposed earlier (Zelcer et al., 2003) it can be envisioned that at lower $E_2 17\beta G$ concentrations the site used for the heterotropic effect is available for the modulators while at greater $E_2 17\beta G$ concentrations it is occupied by the substrate. These data should be taken into consideration when designing drug – transporter interaction assays.

On the basis on these in vitro studies we propose that transporter studies focusing on MRP2/Mrp2 and conducted on rat models would be predictive for humans in most cases. However, the differences in substrate specificity, substrate affinity and regulation of the two transporters might result in significant differences in some cases. As new in vitro methods are available to test the interaction of test drugs with recombinant human transporters, the utilization of these technologies should be considered.

Acknowledgement: This work was also supported by Hungarian Grants GVOP-2004-3.3.2.-2004-04-0001/3.0, GVOP-3.1.1.-2004-05-0506/3.0, EEF-Munka 00034/2003, OTKA T 043141 and FP6-NoE 005137.