Comparison of K_i and IC_{50} Values for Prototypical Inhibitors of the Human ABC Transporters P-gp and BCRP in Membrane Vesicles

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Introduction

P-gp (ABCB1/MDR1) and BCRP (ABCG2) are members of the ATP-binding cassette (ABC) superfamily of transporters that use ATP to actively transport compounds across a cell membrane. P-gp and BCRP are expressed on the luminal membrane of enterocytes, endothelial cells in the brain, the brush border membrane of renal proximal tubules and the canalicular membrane of hepatocytes where they limit intestinal absorption, blood-brain barrier penetration and facilitate excretion into the bile and urine. Compounds that inhibit these transporters may be perpetrators of drug-drug interactions [1,2]. While the FDA Draft Guidance for Industry (2012) recommends IC_{50} values for evaluating the inhibitory potential of transporters, the EMA's Guideline on the Investigation of Drug Interactions (2013) recommends the determination of K_i values [3,4]. The EMA recommends the use of IC_{50} values only when K_i determinations are not possible.

Although K_i values have been reported for inhibitors of various transporters, the methodology differs between labs and oftentimes relies on mathematical extrapolation (*e.g.,* using the IC_{50} value to determine the K_i value with the Cheng-Prusoff equation). In this study the inhibitory potential of various chemical inhibitors was evaluated with *in vitro* experiments in transporter-expressing membrane vesicles to determine both K_i and IC_{50} values. Prior to the conduct of inhibition experiments, K_m values were experimentally determined with each probe substrate, namely N-methylquinidine (NMQ) for P-gp and estrone-3-sulfate (E3S) for BCRP. Subsequently, IC_{50} and K_i values were experimentally determined for the prototypical inhibitors verapamil (P-gp) and Ko143 (BCRP).

Materials & Methods

Materials

AMP, ATP, Ko143, estrone-3-sulfate, $MgCl_2$, MOPS, quinidine, sucrose, Tris-HCl and verapamil were obtained from Sigma (St. Louis, MO, USA). KCl and NaCl were obtained from Fisher (Waltham, MA, USA). [^{3}H]-Estrone-3-sulfate (E3S) was obtained from Perkin Elmer Life and Analytical Sciences (Waltham, MA, USA) or Arc, Inc. (St. Louis, MO, USA). N-Methylquinidine and vesicles expressing MDR1 and BCRP were obtained from Solvo Biotechnology (Szeged, Hungary).

Methods

MDR1 and BCRP vesicles were pre-incubated with or without prototypical inhibitor for 15 min at 37 ± 2°C in incubation buffer based on the manufacturer's protocol [5,6]. After preincubation, probe substrate with AMP or ATP was added and the incubation was continued. For the IC_{50} experiments, the concentration of marker substrate was less than the experimental K_m value. Additionally, K_i experiments were conducted with a range of probe substrate concentrations bracketing the K_m (typically 1/3 to 10-fold K_m). Reactions were terminated by ice-cold washing mix and filtered. The filters were washed five times and then internal standard (MDR1) or scintillation cocktail (BCRP) was added to the plate to lyse the washed vesicles and extract any trapped probe substrate. MDR1 samples were analyzed by LC-MS/MS and BCRP samples were analyzed by liquid scintillation counting (LSC). Experimental conditions are described in Table 1. Two lots of vesicles were evaluated to assess lot-to-lot variation in rates and kinetics.

Table 1. Summary of experimental conditions

Transporter	Experiment	Substrate	Inhibitor	Inc time (min)	Analysis
P-gp	IC_{50}	NMQ (0.3 µM)	Verapamil (0.03, 0.3, 1, 3, 10, 30 µM)	3	LC-MS/MS
	K_i	NMQ (0.3, 0.75, 1.5, 3, 6 µM)	Verapamil (0.03, 0.1, 0.3, 1, 3, 10, 30 µM)	3	LC-MS/MS
BCRP	IC_{50}	H3-E3S (1 µM)	Ko143 (0.025, 0.05, 0.1, 0.15, 0.2, 0.25 µM)	1	LSC
	K_i (low affinity)	H3-E3S (9, 30, 90, 180, 300 µM)	Ko143 (0.025, 0.05, 0.1, 0.2, 0.4, 0.6, 1 µM)	1	LSC
	K_i (high affinity)	H3-E3S (0.5, 1, 2.5, 5, 7.5 µM)	Ko143 (0.025, 0.05, 0.1, 0.15, 0.2, 0.25 µM)	1	LSC

E3S: [3H]-Estrone-3-sulfate *NMQ: N-Methylquinidine* *LSC: Liquid scintillation counting*

Data analysis

IC_{50}, K_m and K_i values were determined with GraFit software (version 7.0.2). K_i values were also calculated from the IC_{50} of the inhibitor and K_m of the probe substrate values using the Cheng-Prusoff equation [7]:

$$K_i = \frac{IC_{50}}{1 + \left(\frac{[S]}{K_m}\right)}$$

Results

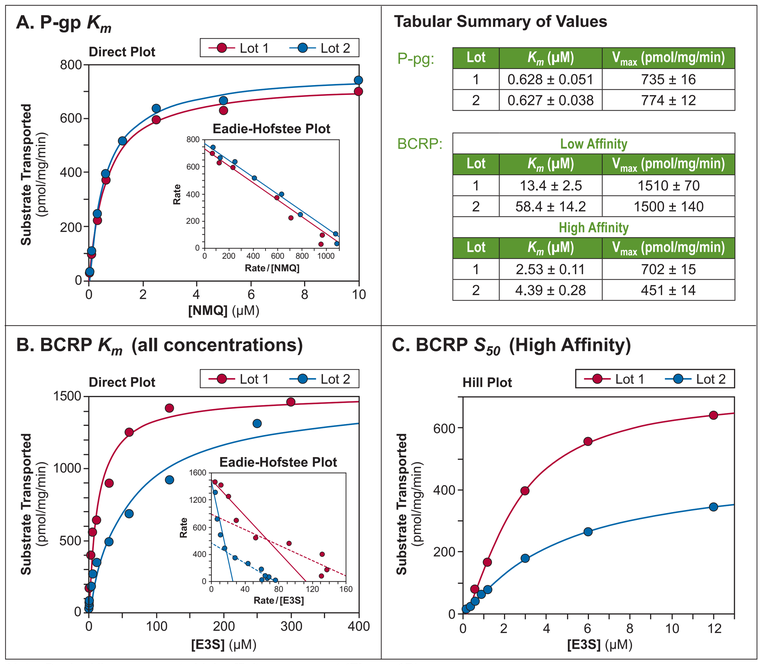
K_m determinations

P-gp: The K_m of NMQ was similar in both lots (0.63 µM) and followed Michaelis-Menten kinetics (Figure 1A).

BCRP: The Eadie-Hofstee plot of E3S activity showed biphasic kinetics. With all E3S concentrations included, the K_m values in lots 1 and 2 were 13 and 58 µM, respectively (Figure 1B). The high affinity component showed allosteric kinetics in both lots and was fit to the Hill equation. The resulting S_{50} values in lots 1 and 2 were 2.5 and 4.4 µM, respectively (Figure 1C).

Results (cont.)

Figure 1. K_m determinations in membrane vesicles



Tabular Summary of Values

P-pg:

Lot	K_m (µM)	V_{max} (pmol/mg/min)
1	0.628 ± 0.051	735 ± 16
2	0.627 ± 0.038	774 ± 12

BCRP:

Low Affinity		
Lot	K_m (µM)	V_{max} (pmol/mg/min)
1	13.4 ± 2.5	1510 ± 70
2	58.4 ± 14.2	1500 ± 140
High Affinity		
Lot	K_m (µM)	V_{max} (pmol/mg/min)
1	2.53 ± 0.11	702 ± 15
2	4.39 ± 0.28	451 ± 14

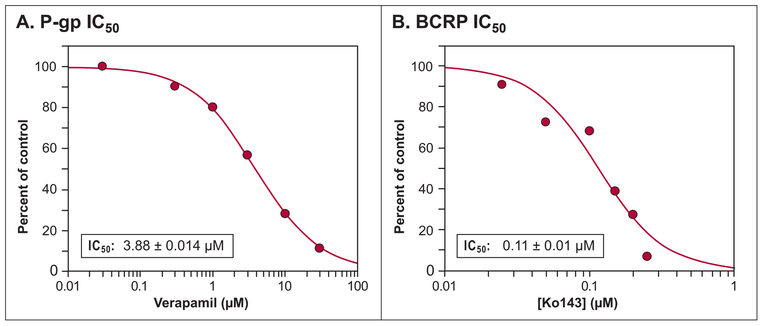
*Note: Dashed line represents high-affinity component (**Figure 1B**)*

IC_{50} determinations

P-gp: Verapamil was incubated with NMQ at 0.3 µM (below the K_m). The resulting IC_{50} value was 3.9 µM (Figure 2A).

BCRP: Ko143 was incubated with E3S at 1 µM (below the K_m and S_{50}). The resulting IC_{50} value was 0.11 µM (Figure 2B).

Figure 2. IC_{50} determinations in membrane vesicles



K_i estimations

P-gp: Using the experimentally determined NMQ K_m (0.63 µM) and verapamil IC_{50} (3.9 µM) in P-gp vesicles, the estimated K_i for verapamil was 2.6 µM.

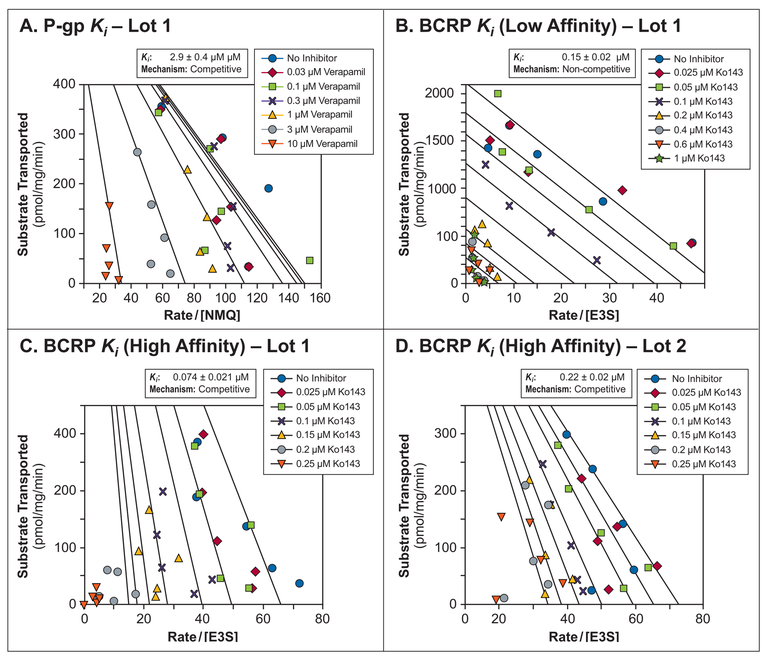
BCRP: Using the experimentally determined E3S K_m (13 µM) or S_{50} (2.5 µM) and Ko143 IC_{50} (0.11 µM) in lot 1 of BCRP vesicles, the estimated K_i for Ko143 was 0.10 or 0.079 µM, respectively.

K_i determinations

P-gp: Verapamil competitively inhibited NMQ accumulation and the K_i value was 2.9 µM (Figure 3A).

BCRP: In lot 1 of BCRP vesicles, the K_i for Ko143 was determined over two concentration ranges of E3S to evaluate the low and high affinity components. Ko143 noncompetitively inhibited E3S in the low affinity concentration range with a K_i value of 0.15 µM (Figure 3B) and in the high affinity concentration range the K_i value was 0.074 µM and was competitive (Figure 3C). In lot 2 of BCRP vesicles, the K_i for Ko143 was determined only for the high affinity concentration range of E3S. The K_i value of Ko143 was 0.22 µM and was competitive (Figure 3D). Attempts were made to fit the high affinity data to multi-site inhibition models but the overall fit did not improve.

Figure 3. K_i determinations in membrane vesicles



Conclusions

P-gp: The lot-to-lot variability of NMQ kinetics in P-gp vesicles was negligible in terms of kinetic parameters and the transport conformed to Michaelis-Menten kinetics.

The experimentally determined IC_{50} and K_i values for verapamil differed by < 2-fold. The estimated and experimentally determined K_i values differed by < 2-fold. The predicted K_i value and experimentally determined IC_{50} and K_i values provided similar predictions of inhibitor potential (within 2-fold).

BCRP: There was notable lot-to-lot variability of E3S kinetics in BCRP vesicles in terms of rate and kinetic parameters. The transport did not conform to simple Michaelis-Menten kinetics but was biphasic. The high affinity component showed allosteric kinetics.

In lot 1, the experimentally determined IC_{50}, low affinity K_i and high affinity K_i values for Ko143 differed by < 2-fold.

In lot 2, the experimentally determined IC_{50} and high affinity K_i values for Ko143 differed by < 2-fold.

Within each lot the experimentally determined IC_{50} and K_i values and predicted K_i value provided similar predictions of inhibitor potential (within 2-fold). However, the K_i values determined in lot 2 were > 2-fold higher than the values determined in lot 1. Lot-to-lot variability should be considered when determining K_i values in transporter vesicles.

Furthermore, the high affinity K_i revealed more potent inhibition and the mechanism of apparent inhibition transitioned from noncompetitive to competitive. The biphasic nature of BCRP-mediated-E3S transport kinetics should be considered when determining K_i values.

References

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