1034

Comparison of K_i and IC_{50} Values for Prototypical Inhibitors of ABC Transporters P-gp and BCRP

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Abstract

Purpose: K_i values were determined for prototypical inhibitors of ABC transporters P-gp and BCRP with an experimental design incorporating multiple substrate and inhibitor concentrations in vesicles. The EMA's Guideline on the Investigation of Drug Interactions (2012) recommends K_i values for evaluating transporter inhibition. The use of IC_{50} values is recommended 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 evaluation of a single probe substrate concentration combined with mathematical extrapolation. These experiments were conducted to compare IC_{50} and K_i values determined in transporter-expressing membrane vesicles.

Methods: Transporter-expressing membrane vesicles were preincubated with a prototypical inhibitor for 15 min at 37±2°C and then a mixture of probe substrate with or without ATP was added and incubated for an additional time point. Terminated reactions were transferred to a filter plate and washed. The filtered vesicles were lysed to extract the probe substrate by either adding internal standard (P-gp) or scintillation fluid (BCRP). Experimental conditions are described in Table 1.

Results: Experimentally determined K_i and IC_{50} values are summarized in Table 2. P-gp and BCRP K_i values were approximately 2-fold higher than the IC_{50} values. Using experimentally determined K_m and IC_{50} values (K_m data not shown), theoretical K_i values were determined using the Cheng-Prusoff equation. Theoretical K_i values for P-gp and BCRP were 0.4 and 0.09 µM, respectively. Compared with the theoretical K_i, the experimentally determined P-gp and BCRP K_i values were 7- and 2-fold higher, respectively.

Conclusions: These results suggest that using experimentally determined K_i values as opposed to the IC_{50} values would not have changed the prediction of inhibitory potential. However, if theoretical K_i values were extrapolated mathematically, inhibition would have been over-predicted in the case of P-gp. It is possible to determine K_i values for P-gp and BCRP in transporter-expressing vesicles; however, the experiments are time-consuming and expensive compared to IC_{50} experiments and may not provide additional value in terms of predicting inhibitory potential.

Background & Purpose

Transporters are proteins located on cell membranes that facilitate the absorption, distribution and excretion of drugs. These transporters are expressed throughout the body. 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 facilitates 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 transporter potential, 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 evaluation of a single probe substrate concentration combined with mathematical extrapolation. These experiments were conducted to compare IC_{50} and K_i values determined in transporter-expressing membrane vesicles.

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. 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 LSC. Experimental conditions are described in Table 1.

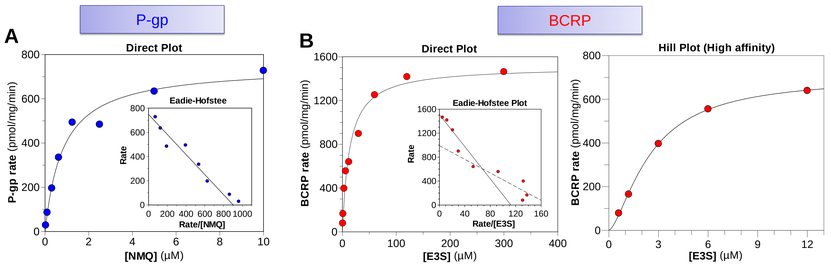
Data analysis: IC_{50}, K_m and K_i values were determined with GraFit software (version 7.0.2). K_i values were also calculated from the IC_{50} values using the Cheng-Prusoff equation[7]: $K_i = IC_{50} \div [1 + ([S] \div K_m)]$

Table 1. Summary of experimental conditions

Transporter	Experiment	Substrate	Inhibitor	Inc time (min)	Analysis
P-gp	IC_{50}	NMQ (2 µM)	Ver (0.01, 0.1, 0.3, 1, 3, 10, 30 µM)	3	LC-MS/MS
	K_i	NMQ (0.3, 0.75, 1.5, 3, 6 µM)	Ver (0.03, 0.1, 0.3, 1, 3, 10, 30 µM)	3	LC-MS/MS
BCRP	IC_{50}	E3S (1 µM)	Ko143 (0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1 µM)	1	LSC
	K_i (low affinity)	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)‡	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; Ver: Verapamil; NMQ: N-Methylquinidine; LSC: Liquid scintillation counting
‡: Experiment added after submission of abstract.

Figure 1. K_m determinations for P-gp and BCRP in membrane vesicles



Transporter	Substrate	K_m (µM) – All concentrations	S_{50} (µM) – High affinity component
P-gp	NMQ	0.83 ± 0.15	NA
BCRP	E3S	13 ± 2	2.5 ± 0.1

Figure 2. IC_{50} determinations for P-gp and BCRP in membrane vesicles

Figure 3. K_i determinations for P-gp and BCRP in membrane vesicles

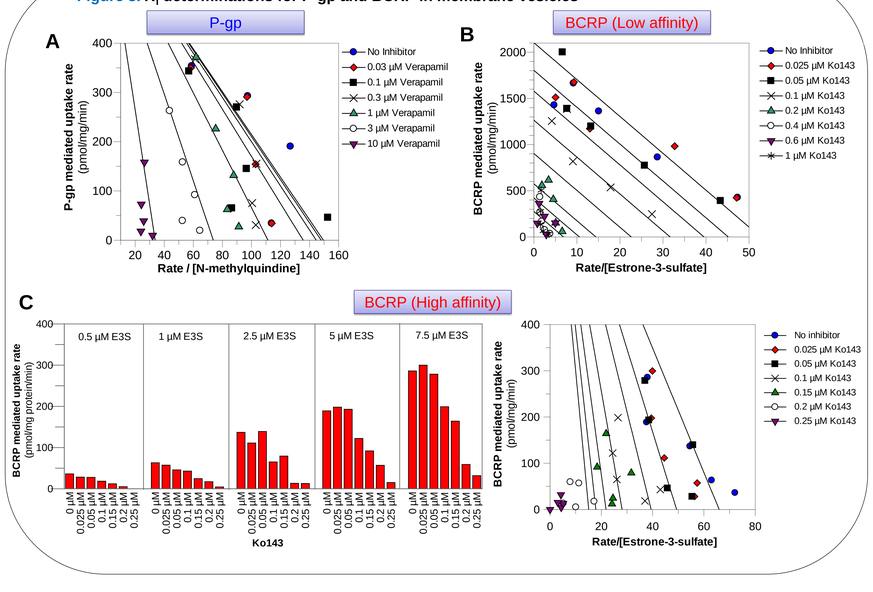


Table 2. Summary of IC_{50} and K_i values

Transporter	Substrate	Inhibitor	IC_{50} (µM)	K_i (µM)	Mechanism (best fit)
P-gp	NMQ	Verapamil	1.2 ± 0.1	2.9 ± 0.4	Competitive
BCRP	E3S	Ko143	0.090 ± 0.013	0.15 ± 0.02 (Low affinity) 0.074 ± 0.021 (High affinity)‡	Noncompetitive Competitive

Results

P-gp and BCRP vesicles were incubated with multiple concentrations of probe substrate to determine the K_m.

- P-gp: The K_m of NMQ was 0.83 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 of E3S was 13 µM. The high affinity component showed allosteric kinetics and the resulting S_{50} was 2.5 µM (**Figure 1B**).

P-gp and BCRP vesicles were incubated with one concentration of probe substrate and multiple concentrations of inhibitor to determine an IC_{50}.

- P-gp: Verapamil was incubated with NMQ at 2 µM (above the K_m). The resulting IC_{50} value was 1.2 µM (**Figure 2A** and **Table 2**).
- BCRP: Ko143 was incubated with E3S at 1 µM (below the K_m and S_{50}). The resulting IC_{50} value was 0.090 µM (**Figure 2B** and **Table 2**).

K_i values were estimated with the Cheng-Prusoff equation.

- P-gp: Using the experimentally determined K_m (0.83 µM) and IC_{50} (1.2 µM), the estimated K_i of verapamil was 0.4 µM.
- BCRP: Using the experimentally determined K_m (13 µM) and IC_{50} (0.09 µM), the estimated K_i of E3S was 0.08 µM. Using the experimentally determined S_{50} (2.5 µM) and IC_{50} (0.09 µM), the estimated K_i of E3S was 0.06 µM.

K_i values were determined experimentally by incubating a range of probe substrate concentrations (spanning the K_m or S_{50} value) with a range of prototypical inhibitor concentrations (spanning the IC_{50} value).

- P-gp: Verapamil competitively inhibited NMQ accumulation and the K_i value was 2.9 µM (**Figure 3A** and **Table 2**).
- BCRP: In the low affinity E3S concentration range (9 to 300 µM) the K_i of Ko143 was 0.15 µM and was noncompetitive (**Figure 3B** and **Table 2**). In the high affinity E3S concentration range (0.5 to 7.5 µM) the K_i of Ko143 was 0.074 µM and was competitive (**Figure 3C** and **Table 2**). Attempts were made to fit the high affinity data to multisite kinetics but the overall fit did not improve.

Conclusions

- P-gp: The experimentally determined K_i and IC_{50} values for verapamil (1.2 and 2.9 µM, respectively) differed by ~2-fold. The estimated and experimentally determined K_i values (0.4 and 2.9, respectively) differed by ~7-fold. The experimentally determined IC_{50} and K_i values provided similar predictions of inhibitor potential (within 2-fold). The estimated K_i over-predicted inhibitory potential.
- BCRP: The experimentally determined IC_{50}, low affinity K_i and high affinity K_i values for Ko143 (0.090, 0.15 and 0.074 µM, respectively) differed by < 2-fold. The estimated low and high affinity K_i values (0.08 and 0.06 µM, respectively) and experimentally determined low and high affinity K_i values (0.15 and 0.074 µM, respectively) also differed by < 2-fold. The experimentally determined IC_{50} and K_i values provided similar predictions of inhibitor potential (within 2-fold). The estimated and experimentally determined K_i values also provided similar predictions (within 2-fold). Although the difference between the low and high affinity K_i values was only 2-fold, the high affinity K_i revealed more potent inhibition and the type of inhibition became competitive. The biphasic nature of ES3 kinetics should be considered when determining K_i values.

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