Comparison of K_i and IC_{50} Values for Prototypical Inhibitors of Uptake Transporters

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Abstract

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Purpose: Ki values were determined for prototypical inhibitors of uptake transporters with an experimental design incorporating multiple substrate and inhibitor concentrations. The EMEA's Guideline on the Investigation of Drug Interactions (2012) recommends that K_i values be calculated for the inhibition of a transporter by investigational drugs and IC_{50} values used only when a K_i value is not possible. Although K_i values have been reported for inhibitors of various uptake transporters, the methodology differs between labs and oftentimes relies on evaluation of a single concentration of the probe substrate combined with a mathematical extrapolation of the K_i value.

Methods: HEK293 cells over-expressing various uptake transporters were cultured in 24-well plates. Cells were pre-incubated with four concentrations of inhibitor (typically 1/6 to 6-fold IC50) for 15 min followed by incubation with a combination of probe substrate (four concentrations; typically 1/3 to 3-fold Km) and inhibitor. Uptake was terminated by aspiration of the incubation media and washing cells three times with cold PBS. For analysis by LC-MS/MS, cells were extracted with 50:50 v/v methanol:water. For analysis by scintillation counting, cells were lysed with 0.1N NaOH. Ki values were determined with GraFit software.

Results: Experimentally determined K_i and IC_{50} values are summarized in the table below. K_i values were similar to IC_{50} values (within two-fold) for inhibition of OATP1B1, OATP1B3, OAT1 and OAT3 by rifampin or probenecid, respectively. However, the K_i value for the inhibition of OCT2 by quinidine was higher than the IC50 value (~two-fold), whereas the K_i value for the inhibition of OCT1 by quinidine was lower than the IC_{50} value (~five-fold).

Introduction

Many clinically-relevant drug uptake transporters are SLC (solute linked carrier transporters) and rely on a concentration gradient of an ion to effect transport of a substrate. These uptake transporters aid in the absorption and distribution of compounds into cells such as hepatocytes (OATP1B1, OATP1B3 and OCT1) and renal proximal tubules (OAT1, OAT3 and OCT2). Compounds that are substrates or inhibitors of transporters may be victims or perpetrators in drug-drug interactions. The EMEA Guideline on the Investigation of Drug Interactions (2012) recommends that K_i values be calculated for the inhibition of a transporter by investigational drugs and IC_{50} values used only when a K_i value is not possible.

Purpose

Although K_i values have been reported for inhibitors of various uptake transporters, methodologies differ between labs and oftentimes rely on evaluation of a single concentration of the probe substrate combined with a mathematical extrapolation of the K_i value. The present study was designed to compare IC_{50} values to K_i values experimentally determined with four concentrations of both probe substrate and inhibitor.

Methods

To determine K_i values, HEK293 cells over-expressing the various uptake transporters were cultured in 24-well plates at a density of 0.2 – 0.4 x 10^6 cells per well in cell culture media for one to three days prior to the experiment. OATP1B1 and OATP1B3 cells were incubated with butyric acid for 24 hours prior to the experiment to inhibit suppression of the transporter. Incubations were carried out in HBSS buffer containing sodium hydrogencarbonate (4 mM) and HEPES (8.4 mM) at pH 7.4. Cells were pre-incubated at 37 ± 2 °C with four concentrations of inhibitor (typically 1/6th to 6-fold IC50) for 15 min followed by incubation with a combination of probe substrate (four concentrations; typically 1/3 – 3-fold K_m) and inhibitor. Conditions are summarized in Table 1. Uptake was terminated by aspiration of the incubation media and washing cells three times with cold PBS. For analysis by LC-MS/MS, cells were extracted with 50:50 v/v methanol:water. For analysis by scintillation counting, cells were lysed with 0.1N NaOH. K_i values were determined with GraFit software (version 7.0.2).

Results

Figure 1. Concentration-dependent inhibition of prototypical substrates of uptake transporters by prototypical inhibitors with in vitro cell-based test systems. Resulting K_i values are summarized in the Table 2.

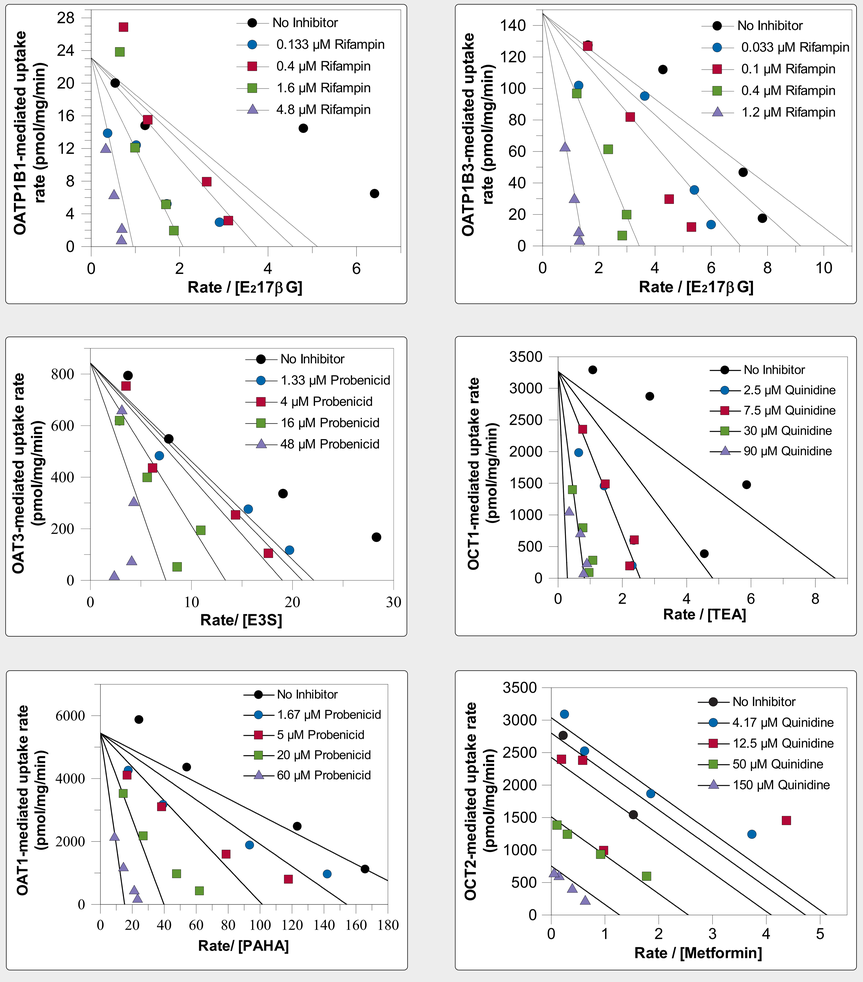

Table 1. Experimental conditions for determining K_i values with four concentrations of both probe substrate and inhibitor.

Transporter	Substrate	Inhibitor	Incubation time (min)	Analysis
OATP1B1	$E_2$17βG (1- 36 µM)	Rif (0.133 - 4.8 µM)	2	LSC
OATP1B3	$E_2$17βG (2.2 - 78 µM)	Rif (0.033 - 1.2 µM)	2	LSC
OAT1	PAHA (6.67 - 240 µM)	Prob (1.67 - 60 µM)	1	LSC
OAT3	E3S (5.83 - 210 µM)	Prob (1.33 - 48 µM)	2	LSC
OCT1	TEA (0.083 - 3 mM)	Quin (2.5 - 90 µM)	15	LC-MS/MS
OCT2	Met (0.33 - 12 mM)	Quin (4.17 - 150 µM)	2	LC-MS/MS

$E_2$17βG: Estradiol-17β-glucuronide; PAHA: p-Aminophippurate; E3S: Estrone sulfate; TEA: Tetraethylammonium bromide; Met: Metformin; Rif: Rifampin; Prob: Probenecid; Quin: Quinidine
LSC: Liquid Scintillation Counting

Table 2. Summary of IC_{50} and K_i values determined for prototypical substrates and inhibitors with in vitro cell-based test systems.

Transporter	Substrate	Inhibitor	IC_{50} (µM)	K_i (µM)	Mechanism (best fit)
OATP1B1	$E_2$17βG	Rifampin	0.80 ± 0.13	1.1 ± 0.6	Competitive
OATP1B3	$E_2$17βG	Rifampin	0.19 ± 0.09	0.19 ± 0.04	Competitive
OAT1	PAH	Probenecid	8.7 ± 3.2	4.7 ± 1.1‡	Competitive
OAT3	E3S	Probenecid	2.8 ± 1.1‡	24.5 ± 8.3‡	Competitive‡
OCT1	TEA	Quinidine	15 ± 3	3.1 ± 1.2	Competitive
OCT2	Metformin	Quinidine	25 ± 1	50 ± 11	Noncompetitive

‡ Experiments were repeated and values are updated from original table in the accepted abstract.

Conclusions

In the present study, the resultant K_i values did not change the prediction of the inhibitory potential compared to the IC_{50} value for three of the six substrate-inhibitor pairs tested. Probe substrate concentrations used in IC_{50} experiments were much lower than K_m and, therefore, the IC_{50} values were expected to be similar to the K_i value. It is possible to determine K_i values in uptake transporter-expressing cells; however, the experiments are time-consuming and expensive compared to IC_{50} experiments. Additionally, given that transporters often follow atypical kinetics and have multiple binding sites makes it difficult to predict K_i values. Further research is recommended to develop robust K_i experimental designs for uptake transporters.

References

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