Introduction

Many clinically-relevant drug-transporter interactions are SLC (solute linked carrier transporters) and rely on a concentration gradient of an ion or effect on 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’s Guideline on the Investigation of Drug Interactions (2012) recommends that IC50 values (within two-fold) for inhibition of OATP1B1, OATP1B3, OAT1 and OAT3 by rifampin or probenecid be calculated for the inhibition of a transporter by investigational drugs and IC50 values used only when a Ki value is not experimentally determined with four concentrations of both probe substrate and inhibitor. The present study was designed to compare IC50 values to Ki values experimentally and oftentimes rely on evaluation of a single concentration of the probe substrate combined with a mathematical extrapolation of the Ki value.

Methods

For analysis by scintillation counting, cells were lysed with 0.1N NaOH. Ki values were determined with GraFit software (version 7.0.2). 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. For analysis by scintillation counting, cells were lysed with 0.1N NaOH. Ki values were determined with GraFit software. For analysis by scintillation counting, cells were lysed with 0.1N NaOH. Ki values were determined with GraFit software. For analysis by scintillation counting, cells were lysed with 0.1N NaOH. Ki values were determined with GraFit software. For analysis by scintillation counting, cells were lysed with 0.1N NaOH. Ki values were determined with GraFit software. For analysis by scintillation counting, cells were lysed with 0.1N NaOH. Ki values were determined with GraFit software. For analysis by scintillation counting, cells were lysed with 0.1N NaOH. Ki values were determined with GraFit software.

Results

In the present study, the resultant Ki values did not change the prediction of the inhibitory potential compared to the IC50 values for the six substrate-inhibitor pairs tested. Probe substrate concentrations used in IC50 experiments often fall outside the range of Ki values for the inhibition of a transporter by investigational drugs and IC50 values used only when a Ki value is not possible.

Purpose

To determine Ki values, HEK293 cells over-expressing the various uptake transporters were cultured in 24-well plates at a density of 2 x 10^5 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 the suppression of the transporter. Incubations were carried out in PBS buffer containing (mM): NaCl (150), KCl (5), glucose (11), MgCl2 (1.13) and HEPES (4.4) 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 13 to 5-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.

References