VARIABILITY OF OATP1B1/1B3 *IN VITRO* INHIBITION CONSTANTS AND THE RESULTING IMPACT ON CLINICAL EVALUATION

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Abstract

The effect of inhibition of the organic anion transporting polypeptides (OATP) 1B1 and 1B3 has continued to grow in clinical significance and recognition. In the last five years, a significant portion of newly approved drugs in the US have been shown to be inhibitors of OATP1B1 and/or OATP1B3 *in vitro*. For this reason, it is critical to understand the effect of experimental variability on drug interaction predictions and how it impacts the decision for a clinical evaluation.

Using the University of Washington Drug Interaction Database (DIDB®), all studies showing *in vitro* inhibition of OATP1B1 and/or -1B3 were identified and those reporting a K_i or IC_{50} value retained. Data were further refined by retaining those inhibitor/substrate pairs with at least three unique experiments reporting an inhibition constant. For each pair, a variability ratio (VR, highest value relative to lowest) was calculated. For OATP1B1, cyclosporine/estradiol-17-β-glucuronide (E217βG) showed the highest variability while rifampin/E217βG showed the most variability for OATP1B3, with VR of 86.4 and 58.1, respectively. Two experimental factors were found to contribute the most to inhibition constant variability – cell type and preincubation versus co-incubation with inhibitors. When these two conditions were accounted for, the overall variability in the entire dataset was reduced from 12.4 to 5.2. A significant degree of substrate-dependency was also observed. Of the inhibitors evaluated, cyclosporine and rifampin were tested with the largest array of substrates, and interestingly, the highest variability was observed with non-clinically relevant substrates, namely E217βG, estrone-3-sulfate, and bromosulfophthalein (BSP). For OATP1B1, cyclosporine VR ranged from 3.4 to 86.3 (atorvastatin and E217βG, respectively) while rifampin VR ranged from 3.9 to 43.6 (atorvastatin and BSP, respectively). When only HEK293 cells and co-incubation were considered, the variability for each inhibitor was reduced to a range of 3.0 – 12.6 for cyclosporine and 3.9 – 7.9 for rifampin.

To determine the effect of the observed variability on clinical predictions, R-values (as described in the latest FDA guidance) were calculated for each constant and the range for each inhibitor determined. Despite significant changes in VR when incubation conditions were accounted for, the resulting R-values did not show a significant shift with respect to the FDA cut-off value for prompting a clinical evaluation (R ≥ 1.1). For the recommended index inhibitors cyclosporine and rifampin, all calculated R-values were ≥ 1.1 regardless of the *in vitro* conditions. Similar to VR, R-values showed substrate-dependent variability with the fold-change within each inhibitor/substrate pair ranging from 2.3 – 51.2 for cyclosporine and 3.1 – 12.8 for rifampin. This variability, both within the pairs and for the inhibitors overall, was decreased when the two primary sources of variability were accounted for, ranging from 2.3 – 8.6 for cyclosporine and 2.8 – 5.7 for rifampin.

These results suggest significant variability in *in vitro* inhibition constants for inhibitors of OATP1B1 and OATP1B3, which translates to variability in R-values, highlighting the importance of standardizing the experimental conditions, including the index substrate used.

Methods

- All studies available in the DIDB® showing *in vitro* inhibition of OATP1B1 and/or OATP1B3 were collected, and those studies providing K_i and/or IC_{50} data were retained.
  - Experiments with K_i values were analyzed separately from those with IC_{50} values for consistency of data.
- Data was collated by inhibitor/substrate pair (ISP) and those with a minimum of three experimental results were analyzed by calculating variability ratios (VR – highest value relative to lowest for a given dataset).
  - VR were calculated for all data for each ISP as well as after considering key experimental factors to evaluate sources of variability.
- To determine the effect of *in vitro* variability on DDI predictions, R-values were calculated, and ranges were determined for the full datasets as well as for individual experimental conditions.

Due to the small dataset available for evaluation, only a descriptive analysis was able to be completed.

Results

A total of 128 studies from 44 publications were examined (*Table 1*)

- For both OATP1B1 and 1B3, estradiol-17-β-glucuronide ($E_2$17βG) was the most common substrate (62% of all studies); rifampin (27%) and cyclosporine (25%) were the most commonly used inhibitors.

Results (cont.)

Most studies were completed in HEK293 cells (79%)

Table 1. Identified in vitro inhibitor/substrate pairs (ISPs) for evaluation

	ISPs with ≥ 3 Studies	
	IC_{50}	K_i
OATP1B1	21	7
OATP1B3	2	--

IC_{50} Variability

Both OATP1B1 and 1B3 showed high IC_{50} variability and lower VRs were observed for the K_i values.

Table 2. Highest calculated variability ratios (VRs) by transporter and inhibition constant

	Highest VR	
	IC_{50}	K_i
OATP1B1	86.4 (CsA/$E_2$17βG, n = 11)	7.2 (GEM/$E_2$17βG; n = 3)
OATP1B3	58.2 (RIF/$E_2$17βG, n = 7)	--

CsA- cyclosporine; $E_2$17βG – estradiol-17-β-glucuronide; GEM- gemfibrozil; RIF- rifampin.

Cell type and preincubation versus co-incubation were found to contribute the most to inhibition constant variability (*Figure 1*).

- Mean VR for all OATP1B1 IC_{50} ISPs was reduced from 11.7 to 8.5 and 7.3 when only HEK293 cells and co-incubation were considered, respectively
  - Further reduced to 4.2 when both factors were considered together.
- VR for OATP1B1 K_i values was reduced from 3.8 to 2.0
- OATP1B3 IC_{50} mean VR showed a similar trend but did not have sufficient data to evaluate the contribution of cell type.

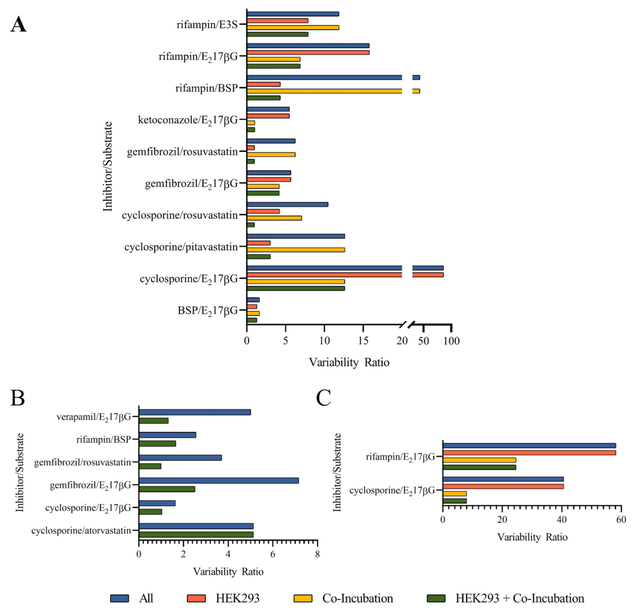

Figure 1. Calculated ISP variability ratios (VRs) for OATP1B1 IC_{50} (A) and K_i (B) values as well as OATP1B3 IC_{50} values (C). None of the identified Ki experiments used preincubation, therefore only the effect of cell type is presented. VR for all studies (blue), only HEK293 cells (orange), only co-incubation (yellow), and HEK293 with co-incubation (green).

Choice of substrate was also found to have an effect on VR with the largest variability observed for non-clinically relevant substrates; reduced when experimental conditions were accounted for

Table 3. Variability ratios before and after correction for clinical and non-clinical substrates

		Variability Ratio (VR)			
Inhibitor	Substrate	All Data	HEK293	Co-Incubation	HEK293 + Co-Incubation
Cyclosporine	$E_2$17βG	86.3	86.3	12.6	12.6
	Pitavastatin	12.7	3.0	12.7	3.0
Rifampin	BSP	43.6	4.3	43.6	4.3
	Atorvastatin	3.9	3.9	3.9	3.9

R-Value Variability

To determine the effect of the observed variability on clinical predictions, R-values were calculated for each constant and the range and fold-change was determined for each ISP, as well as each inhibitor overall

Despite significant changes in VR, resulting R-values did not show a significant shift relative to the FDA clinical evaluation cut-off value of 1.1 (*Figure 2*)

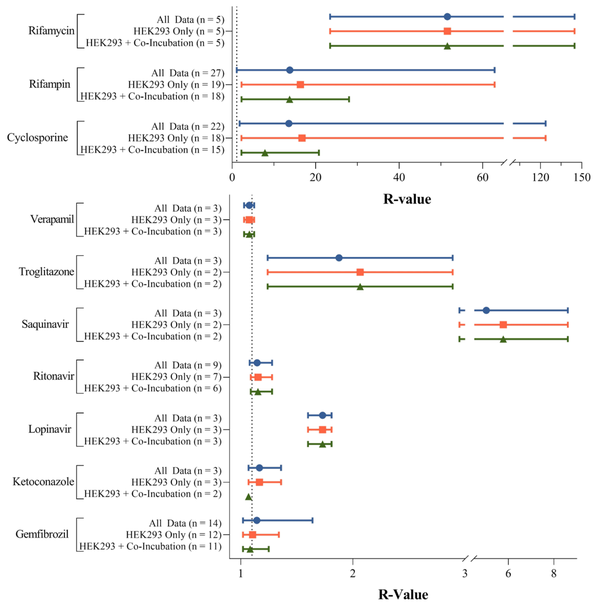

Figure 2. Effect of experimental conditions on R-value. For each inhibitor, the mean R-value and range for each subset is presented, with the number of studies (n) in parenthesis. The vertical dashed line indicates the FDA cut-off value of 1.1. The blue lines are all collected data, orange lines are experiments performed in HEK293 cells only, and green lines HEK293/co-incubation only.

Conclusions

- The descriptive analysis performed herein evaluated a broad dataset, identifying two main areas of experimental design that significantly contributed to IC_{50} and K_i variability – cell system and preincubation with the inhibitor
  - Accounting for these factors dramatically reduced observed variability
  - Choice of substrate also influenced inhibitor constant variability
- Current experimental design is moving towards controlling for these parameters
  - The 2017 FDA guidance on *in vitro* assessment of DDIs recommends a 30-minute preincubation period
  - Despite a lack of written guidance on cell type, 80% of experiments in the last five years used HEK293 cells
- Choice of probe substrate influenced variability, with a broader range of inhibitor constants reported with *in vitro* probes compared to clinical substrates
- The preclinical variability did not appear to affect *in vitro* to *in vivo* predictions for the inhibitors evaluated, as almost all calculated R-values were above the FDA cut-off value
- It is important to note that this descriptive analysis was limited by the availability of literature data regarding *in vitro* OATP1B1/1B3 inhibition
  - It is possible that the conclusions reached here may underestimate the variability in OATP1B1/1B3 inhibitory constants, and subsequently R-values, as drugs available for analysis with ≥ 3 published values are marker inhibitors recommended by the FDA