# Comparison of $K_i$ and $IC_{50}$ Values for Prototypical and Clinically-Relevant Probe Substrates of the Hepatic Transporters OATP1B1 and OATP1B3

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## Introduction

Organic anion transporting polypeptides 1B1 and 1B3 (OATP1B1, *SLCO1B1* and OATP1B3, *SLCO1B3*) are transmembrane proteins expressed in the sinusoidal membrane of human hepatocytes and are capable of transporting a wide range of hydrophilic molecules from the blood into the hepatocyte for subsequent metabolism and/or elimination. Inhibition of OATP1B1 and/or OATP1B3 by xenobiotics can lead to pharmacokinetic drug-drug interactions whereby the exposure (AUC) of a co-administered victim drug (substrate) is increased in the presence of the inhibitory drug. For example, orally administered cyclosporin inhibits OATP-mediated uptake of pravastatin-, a prototypical *in vivo* probe substrate of OATP, which causes a 9.9-fold increase in AUC.[1] The increase in systemic exposure to narrow safety margin drugs (e.g. statins) results in undesired side-effects and dose-limiting toxicities. Therefore, the FDA and EMEA recommend the evaluation of new drug candidates for the potential to inhibit OATP1B1 and OATP1B3 which is often first evaluated with *in vitro* test systems, typically $IC_{50}$ experiments.

The recent EMEA Guideline on the Investigation of Drug Interactions (2012) recommends the calculation of $K_i$ values when evaluating the inhibition of a transporter by investigational drugs.[2] Although $K_i$ values have been reported for inhibitors of various uptake transporters, the difficulty of experimental design coupled with vigorous mathematical extrapolation can make the determination of intrinsic $K_i$ values difficult and oftentimes reverts to the determination of extrinsic $IC_{50}$ values. Additionally, it is important to consider clinically relevant substrates rather than prototypical probe substrates when evaluating potential DDIs of investigational drugs at the transporter level. In this study, $K_i$ and $IC_{50}$ values were determined for one prototypical (estradiol-17β-glucuronide (E2G)) and one clinically-relevant (pravastatin) substrate of the hepatic uptake transporters OATP1B1 and OATP1B3 with an experimental design incorporating multiple substrate and inhibitor concentrations.

## Methods

HEK293 cells over-expressing either OATP1B1 or OATP1B3 were obtained from Sekisui Medical Division, LTD (Tokai, Japan). Pravastatin and rifampin were obtained from Sigma (St. Louis, MO, USA). [$^3$H]Estradiol-17β-glucuronide (E2G) was obtained from Perkin Elmer Life and Analytical Sciences (Waltham, MA, USA).

In brief, HEK293 cells over-expressing OATP1B1 or OATP1B3 were cultured in 24-well plates. Cells were pre-incubated (15 min at 37°C) with four concentrations of the inhibitor, rifampin, at concentrations bracketing the experimentally determined $IC_{50}$ value (typically 1/6 to 6-fold the experimentally determined $IC_{50}$). The pre-incubation was followed by incubation (1 min) with six concentrations of substrate (typically 1/3 to 3-fold the experimentally determined $K_m$) and inhibitor. Uptake was terminated by washing the cells three times with cold 1x PBS. For analysis by scintillation counting (E2G), cells were lysed with 0.1 M NaOH. For analysis by LC-MS/MS (pravastatin), cells were extracted with 50:50 v/v methanol:water. $IC_{50}$ values were determined with GraphPad Prism software (version 6.01). $K_m$ and $K_i$ values were determined with GraFit software (version 7.0.2).

## Results

**Figure 1.** Experimentally determined inhibition profiles ($IC_{50}$ and $K_i$) of OATP1B1-mediated uptake of E2G and pravastatin into HEK-OATP1B1 cells by rifampin

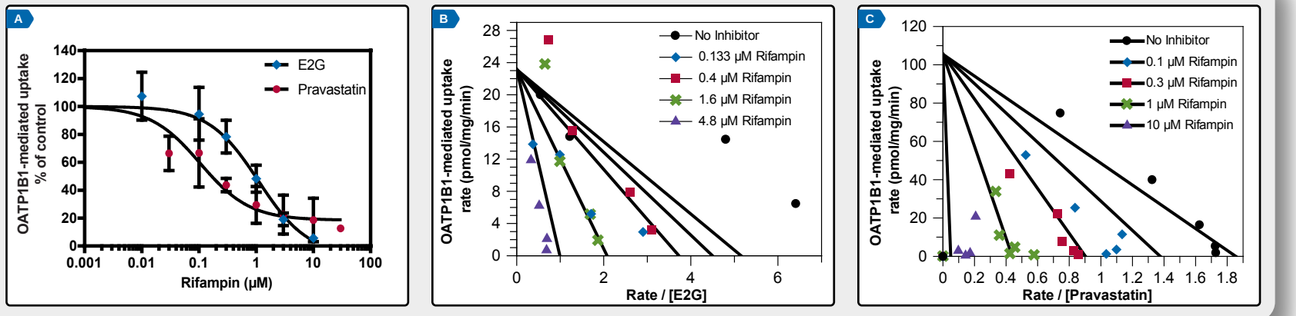


**Figure 2.** Experimentally determined inhibition profiles ($IC_{50}$ and $K_i$) of OATP1B3-mediated uptake of E2G and pravastatin into HEK-OATP1B3 cells by rifampin

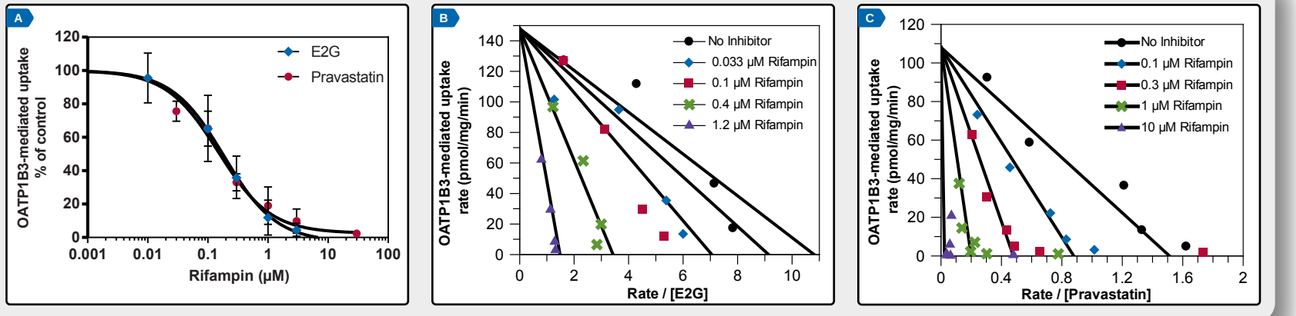


**Table 1**. Summary of experimentally-determined $IC_{50}$ and $K_i$ values for the inhibition of OATP1B1- and OATP1B3-mediated uptake by rifampin

| Transporter | Substrate | Inhibitor | $K_m$ (μM) | $IC_{50}$ (μM) | $K_i$ (μM) | Mechanism (best fit) |
|---|---|---|---|---|---|---|
| **OATP1B1** | E2G | Rifampin | 4.5 ± 1.9 | 0.79 ± 0.13 | 1.1 ± 0.6 | Competitive |
| **OATP1B3** | E2G | Rifampin | 13.7 ± 7.7 | 0.19 ± 0.09 | 0.19 ± 0.04 | Competitive |
| **OATP1B1** | Pravastatin | Rifampin | 56.8 ± 15.7 | 0.16 ± 0.02 | 0.28 ± 0.08 | Competitive |
| **OATP1B3** | Pravastatin | Rifampin | 71.6 ± 10.7 | 0.25 ± 0.05 | 0.14 ± 0.02 | Competitive |

- **Figure 1** illustrates the inhibition of OATP1B1-mediated uptake of E2G and pravastatin by rifampin. Rifampin inhibited E2G and pravastatin uptake into OATP1B1-expressing cells with $IC_{50}$ values of 0.79 and 0.16 μM, respectively (**Figure 1A and Table 1**). Rifampin inhibited the uptake of E2G and pravastatin with $K_i$ values of 1.1 and 0.28 μM, respectively (**Figure 1B and 1C**).
- Experiments conducted with E2G as a probe substrate for OATP1B1 yielded comparable $K_i$ and $IC_{50}$ values (1.1 and 0.79 μM, respectively). Similar results were observed with the clinically-relevant probe substrate pravastatin ($K_i$ and $IC_{50}$ values of 0.28 and 0.16 μM, respectively).
- **Figure 2** illustrates the inhibition of OATP1B3-mediated uptake of E2G and pravastatin by rifampin. Rifampin inhibited E2G and pravastatin uptake into OATP1B3-expressing cells with $IC_{50}$ values of 0.19 and 0.25 μM, respectively (**Figure 2A and Table 1**). Rifampin inhibited the uptake of E2G and pravastatin with $K_i$ values of 0.19 and 0.14 μM, respectively (**Figure 2B and 2C**).
- Similar to the observed results for OATP1B1, experiments conducted with E2G as a probe substrate for OATP1B3 yielded comparable $K_i$ and $IC_{50}$ values (0.19 and 0.19 μM, respectively). Also, similar results were observed with the clinically-relevant probe substrate pravastatin ($K_i$ and $IC_{50}$ values of 0.14 and 0.25 μM, respectively).
- However, substrate-dependent inhibition was observed in OATP1B1 cells but not in OATP1B3 cells; rifampin inhibited OATP1B1-mediated pravastatin uptake with approximately four-fold more potency than observed with E2G with $K_i$ values of 1.1 and 0.28 μM, respectively (**Table 1**).
- In all cases, the mechanism of inhibition for OATP1B1- and OATP1B3-mediated uptake of E2G and pravastatin by rifampin was determined to be competitive according to the best fit of the experimental data (**Table 1**).

## Conclusions

- 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.
- Overall, $K_i$ values were similar to $IC_{50}$ values (within two-fold) for inhibition of OATP1B1 and OATP1B3.
- Substrate-dependent inhibition by rifampin was observed in OATP1B1 cells but not in OATP1B3 cells; rifampin inhibited OATP1B1-mediated pravastatin uptake with approximately four-fold more potency than observed with E2G.
- In some cases, experimentally determined $K_i$ values may be more accurate for DDI predictions than $IC_{50}$ values, and, in some cases, should be determined with a clinically relevant substrate.

## References

1. Zhang L et al. (2011) Clin Pharmacol Ther. **89**: 481-484.
2. [EMA] European Medicines Agency. EMA/CHMP/EWP/125211/2012. Guideline on the Investigation of Drug Interactions, Committee for Human Medicinal Products (CHMP), London, England.