

# 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.<sup>1</sup> 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.<sup>2</sup> 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-17 $\beta$ -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-17 $\beta$ -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

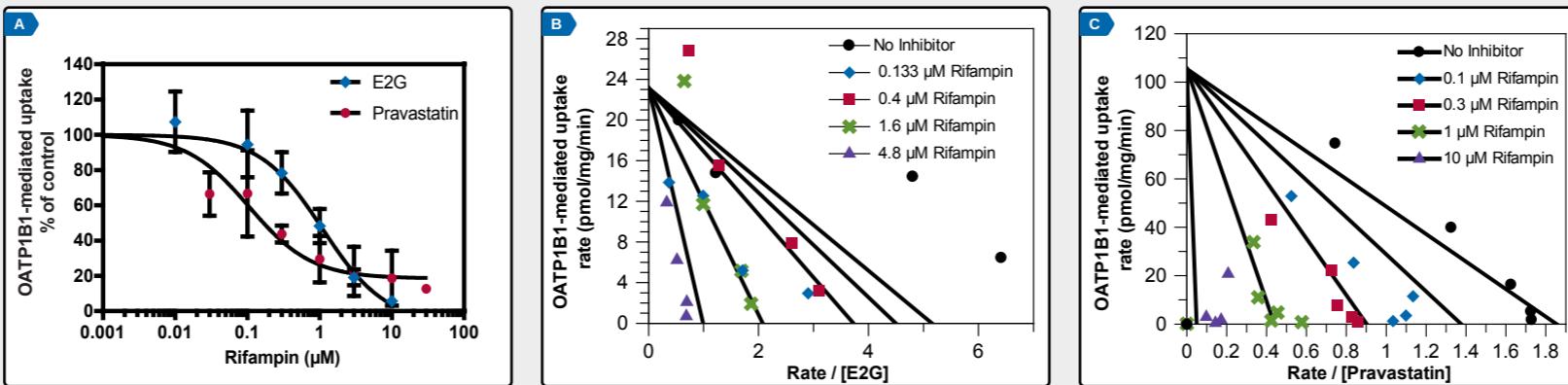


Figure 2. Experimentally determined inhibition profiles ( $IC_{50}$  and  $K_i$ ) of OATP1B3-mediated uptake of E2G and pravastatin into HEK-OATP1B3 cells by rifampin

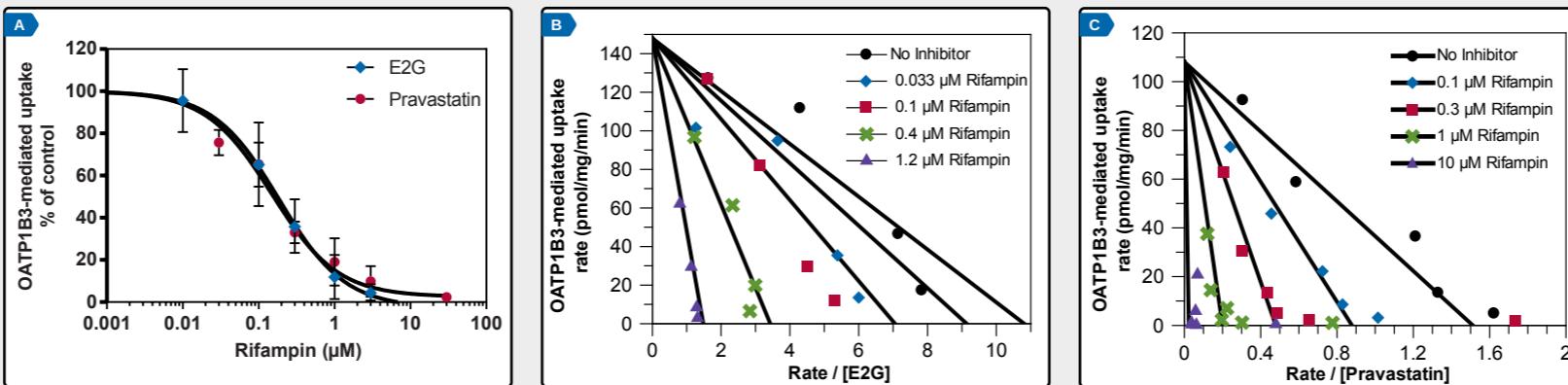


Table 1. Summary of experimentally-determined  $IC_{50}$  and  $K_i$  values for the inhibition of OATP1B1- and OATP1B3-mediated uptake by rifampin

Transporter	Substrate	Inhibitor	$K_m$ ( $\mu$ M)	$IC_{50}$ ( $\mu$ M)	$K_i$ ( $\mu$ M)	Mechanism (best fit)
OATP1B1	E2G	Rifampin	$4.5 \pm 1.9$	$0.79 \pm 0.13$	$1.1 \pm 0.6$	Competitive
OATP1B3	E2G	Rifampin	$13.7 \pm 7.7$	$0.19 \pm 0.09$	$0.19 \pm 0.04$	Competitive
OATP1B1	Pravastatin	Rifampin	$56.8 \pm 15.7$	$0.16 \pm 0.02$	$0.28 \pm 0.08$	Competitive
OATP1B3	Pravastatin	Rifampin	$71.6 \pm 10.7$	$0.25 \pm 0.05$	$0.14 \pm 0.02$	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  $\mu$ M, respectively (Figure 1A and Table 1). Rifampin inhibited the uptake of E2G and pravastatin with  $K_i$  values of 1.1 and 0.28  $\mu$ M, 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  $\mu$ M, respectively). Similar results were observed with the clinically-relevant probe substrate pravastatin ( $K_i$  and  $IC_{50}$  values of 0.28 and 0.16  $\mu$ M, 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  $\mu$ M, respectively (Figure 2A and Table 1). Rifampin inhibited the uptake of E2G and pravastatin with  $K_i$  values of 0.19 and 0.14  $\mu$ M, 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  $\mu$ M, 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  $\mu$ M, 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  $\mu$ M, 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

- Zhang L et al. (2011) Clin Pharmacol Ther. 89: 481-484.
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