OBSERVED VS. PREDICTED EFFECTS OF PROTEIN BINDING: IMPROVING *IN VIVO* PREDICTIONS OF HEPATIC CLEARANCE, IC_{50} VALUES, AND INTRACELLULAR CONCENTRATIONS.

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Introduction

The unbound intracellular concentration is the common integrative factor in relating *in vitro* results to *in vivo* observations. *In vitro* studies are often performed in the absence of protein, and the extent of protein binding (determined in a separate experiment under equilibrium conditions) is then applied to translate the data for prediction of *in vivo* effects. If active transport processes are involved in hepatic uptake, intracellular concentrations (bound and free) can differ greatly from the concentration outside of the cell. *In vivo* the kinetics (i.e. on and off rates) of the binding interactions are also important, since it is the rate limiting step of the overall uptake process that will determine the uptake of a drug. We studied the effects of protein on the transport and metabolic parameters in *in vitro* systems to evaluate these effects.

Methods

Human Hepatocytes Rat hepatocytes (Wistar) or cryopreserved, TRANSPORTER CERTIFIED® human hepatocytes in a sandwich configuration (SCHH) were cultured using QUALGRO™ Media for 4 or 5 days, respectively.

Treatments The accumulation of carboxydichlorofluorescien (CDF) in bile canaliculi from sandwich-cultured rat hepatocytes was measured following exposure to carboxydichlorofluorescien-diacetate (CDF-DA) in the absence or presence of a physiological concentration of bovine serum albumin (4% BSA). On Day 4 (rat) or day 5 (human) of culture, hepatocyte cultures were exposed to test compounds. Sandwich-cultured rat hepatocytes and B-CLEAR® technology were used to determine the intracellular concentration (ICC) and biliary clearance (CIbiliary) for 10 compounds in the presence and absence 4% BSA. In separate studies, the effects of human serum albumin and rat serum were also evaluated.

Increasing concentrations of Telmisartan (0.01-20 µM) were incubated with SCHH in the presence and absence of 4% BSA to evaluate the effect on the biliary clearance of taurocholate. Telmisartan is highly protein bound (99.5%) to albumin.

IC50 values for fluconazole and ketoconazole were determined in SCHH in the presence and absence of protein (4% BSA) using midazolam as the probe substrate. The IC50 value determined in the presence of 4% BSA (Observed IC50) was compared to the Predicted IC50 calculated by multiplying the IC50 determined in the absence of 4% BSA by the fraction unbound (fu).

The extent of protein binding in 4% BSA was determined using 96 well equilibrium dialysis and used to normalize intracellular concentration and clearance values obtained in the absence of protein (Predicted value), compared to the value obtained in the presence of protein (Observed value) and the fold change was calculated.

Results

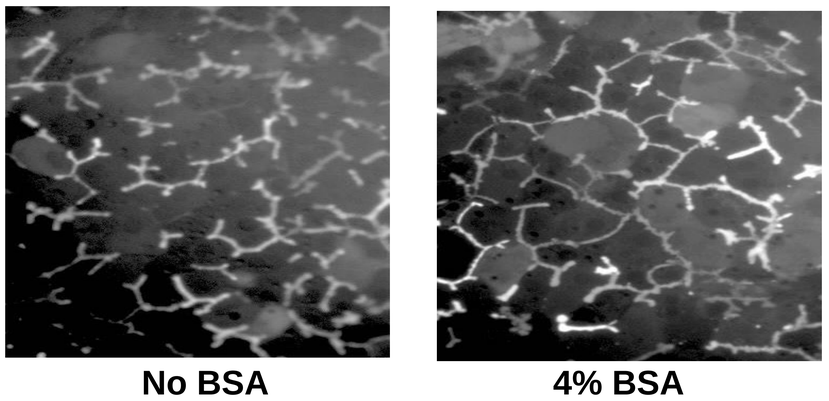


Figure 1 CDF-diacetate is taken up by passive diffusion and cleaved to CDF. Addition of BSA had no discernable effects on CDF accumulation in bile canalicular networks*

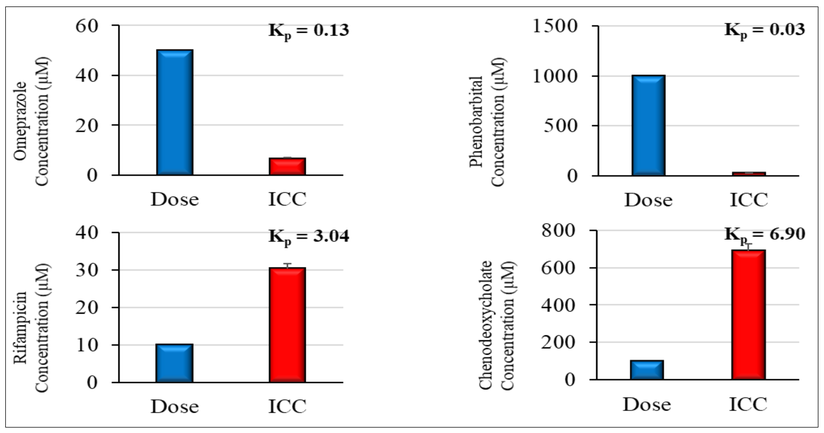


Figure 1 Dose concentration vs. Intracellular concentration (ICC) of selected metabolic and transporter inducers measured in the presence of 4% BSA. Accumulation (Kp) = ICC/Dose Concentration.

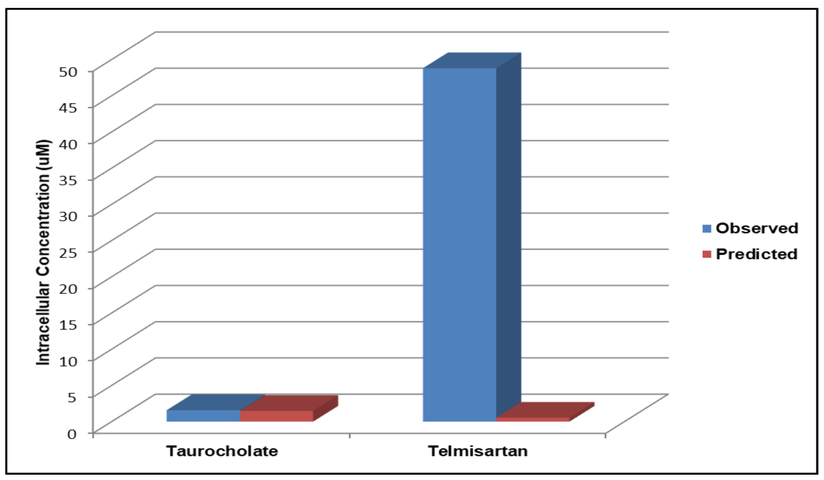
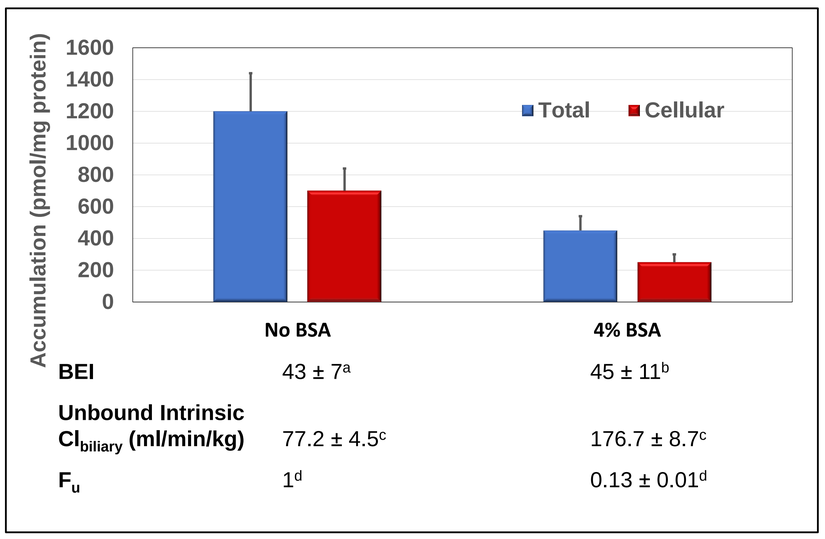


Figure 2 **Observed** ICC values in the presence of 4% BSA compared to ICC values **Predicted** from the fraction bound and values generated in the absence of protein. Taurocholate (80% bound) ICC in the presence of protein was predicted from *in vitro* data generated in the absence of protein. Telmisartan (99% bound) intracellular concentration was underpredicted by 90X.



	No BSA	4% BSA
BEI	43 ± 7[a]	45 ± 11[b]
Unbound Intrinsic $Cl_{biliary}$ (ml/min/kg)	77.2 ± 4.5[c]	176.7 ± 8.7[c]
F_u	1[d]	0.13 ± 0.01[d]

Figure 3 Rosuvastatin hepatic accumulation (Total and Cellular), biliary excretion index (BEI), unbound intrinsic CIbiliary, and Fu in the absence or presence of 4% BSA in sandwich-cultured rat hepatocytes. n=3 livers in triplicate. Data are presented and mean ± SEM. Groups with the same letter indicate a statistically significant difference ($p<0.05$).

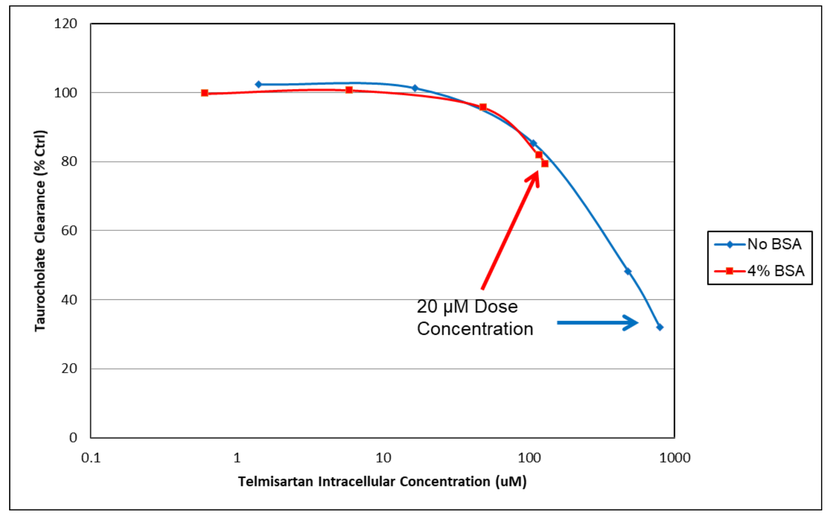


Figure 4 Inhibition of taurocholate biliary clearance as a function of the intracellular concentration of telmisartan in SCHH. Inhibition of taurocholate biliary clearance depends only on the intracellular concentration of telmisartan,

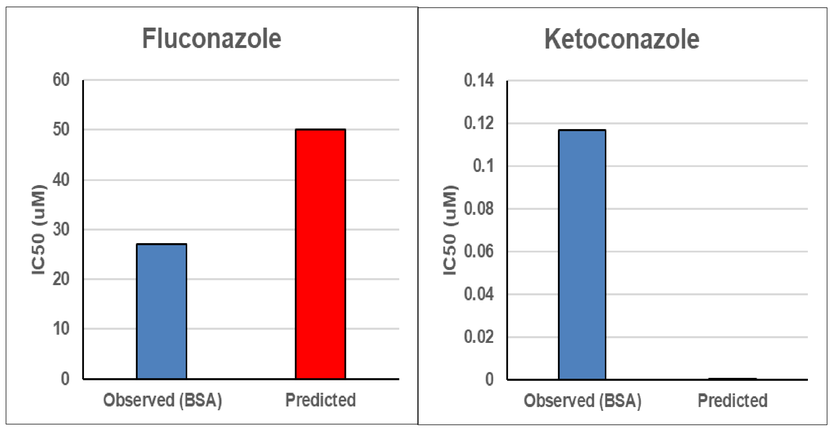


Figure 5 Effect of protein on the estimation of IC50 values for CYP2C9 and CYP3A4 inhibition. IC50 values for inhibition of CYP2C9 inhibition by fluconazole were over predicted by 2X. IC50 values for inhibition of CYP3A4 by ketoconazole were under predicted by 257X.

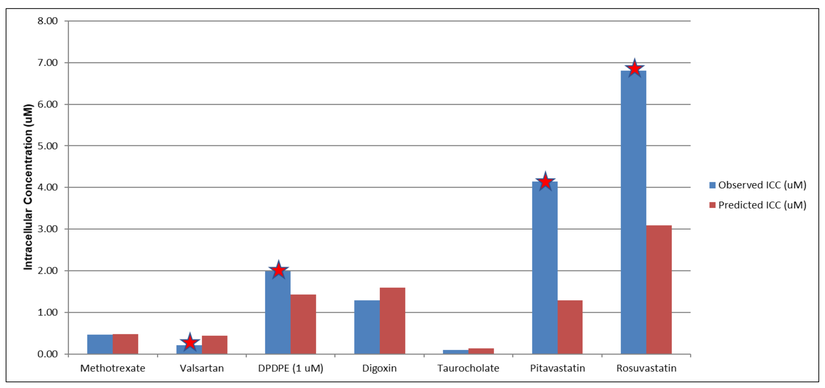


Figure 6 Comparison of intracellular concentrations (Total) for selected compounds determined from TRANSPORTER CERTIFIED™, human hepatocytes in sandwich-culture using B-CLEAR® technology, in the absence and presence of 4% BSA. The intracellular concentration (ICC) was underpredicted for pitavastatin (3.2-fold), rosuvastatin (2.2-fold), and telmisartan (90-fold) when the study was not performed in the presence of a physiological concentration of protein. Over prediction of the ICC for valsartan (2.1-fold) was also observed.

Conclusions

The **LACK** of agreement between observed and predicted intracellular concentrations may be due to measurement of the EXTENT and not the AFFINITY of the protein binding.

Addition of physiologic protein concentrations to *in vitro* systems may improve predictions of a drug's *in vivo* properties and effects.

For a copy of this poster or additional questions, contact:

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* Wolf, K.K. Effect of Albumin on the Biliary Clearance of Compounds in Sandwich-Cultured Rat Hepatocytes. DMD 36:2086-2092, 2008