IMPROVING THE PREDICTION OF HEPATOTOXICITY: IMPACT OF PROTEIN BINDING IN THE GENERATION OF *IN VIVO* RELEVANT INTRACELLULAR CONCENTRATIONS.

Kenneth R. Brouwer, Jonathan P. Jackson, Robert L. St. Claire III, and Ronald Laethem BioIVT, ADME-TOX Division, 2810 Meridian Parkway, Suite 100, Durham, NC 27713

Introduction

The unbound intracellular concentration (ICC) is the driving force for processes that occur inside the hepatocyte, including metabolism, induction (metabolic and transporter), efflux-based drug interactions, and hepatotoxicity. In sandwich-cultured hepatocytes, the intracellular milieu contains the components for drug binding, and in concert with hepatic uptake and efflux (basolateral and canicular) transporters, drug metabolizing enzymes, and key regulatory pathways allow generation of in vivo relevant unbound intracellular concentrations. Protein on the outside of the cell can also limit hepatic uptake of a drug. If hepatic uptake and intracellular concentration are dependent on the free concentration, then, parameters generated from experiments performed in the absence and presence of protein should be equal when normalized for protein binding.

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 carboxydichlorofluorescein (CDF) in bile canaliculi from sandwich-cultured rat hepatocytes was measured following exposure to carboxydichlorofluorescein-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. 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. Sandwich-cultured rat hepatocytes and B-CLEAR® technology were used to determine the intracellular concentration (ICC) and biliary clearance ($Cl_{biliary}$) 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.

IC_{50} 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 IC_{50} value determined in the presence of 4% BSA (**Observed** IC_{50}) was compared to the **Predicted** IC_{50} calculated by multiplying the IC_{50} 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

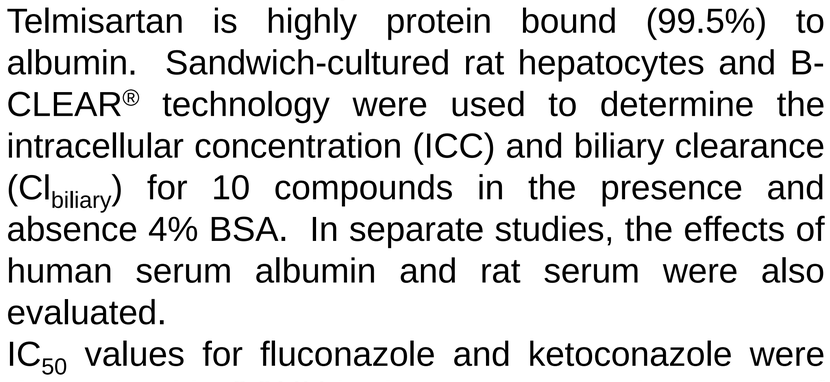

Figure 1 CDF-diacetate is taken up by passive diffusion and cleaved to CDF. Addition of BSA had no discernable effects on CDF accumulation in bile canalicular networks*

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

For a copy of this poster or additional questions, contact:
Kenneth Brouwer at (919) 593-2519 or KBrouwer@BioIVT.com

Table 1 Intracellular concentration (ICC) and hepatic accumulation (Kp) in sandwich-cultured human hepatocytes in the presence of 4% BSA. Hepatic accumulation (Kp) = ICC/Dose Concentration. If transporters are required for the uptake of a drug, the intracellular concentration (ICC) of the drug can differ greatly from the extracellular concentration.

	Dose Concentration (uM)	Intracellular Concentration (uM)	Kp Ratio
Telmisartan	1	36.4	36
Cyclosporine	1	28	28
Bosentan	36	66.0	2
Rosuvastatin	10	11.5	1

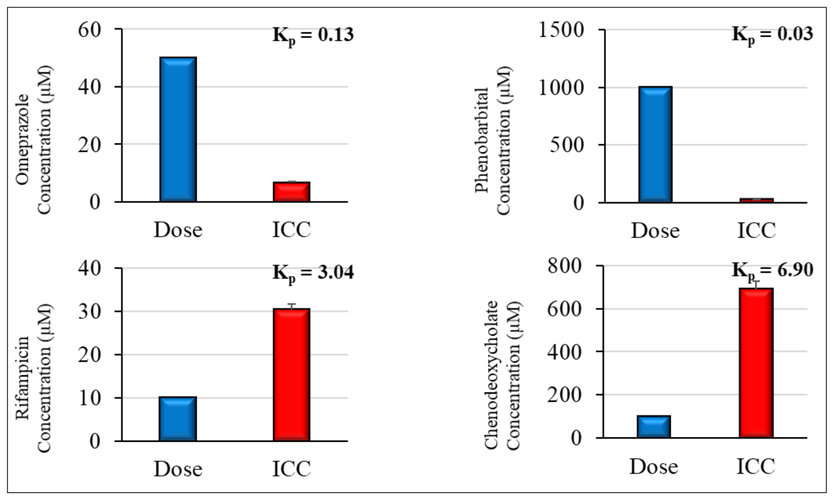

Figure 2 Dose concentration vs. Intracellular concentration (ICC) of selected metabolic and transporter inducers measured in the presence of 4% BSA. Accumulation (Kp) was determined by dividing the ICC by the dose concentration. Measured intracellular concentrations (total) may be very different than concentrations inside the hepatocyte when transporters are involved in the uptake of compounds.

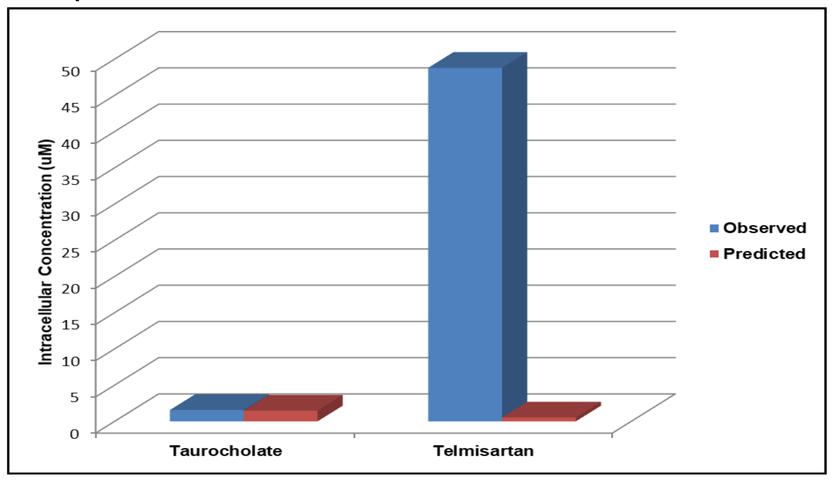

Figure 3 **Observed** ICC values in the presence of 4% BSA compared to ICC values **Predicted** from the fraction bound and values generated in the absence of protein. Taurocholate (80% bound) ICC in the presence of protein was predicted from *in vitro* data generated in the absence of protein. Telmisartan (99% bound) intracellular concentration was **underpredicted** by 90X.

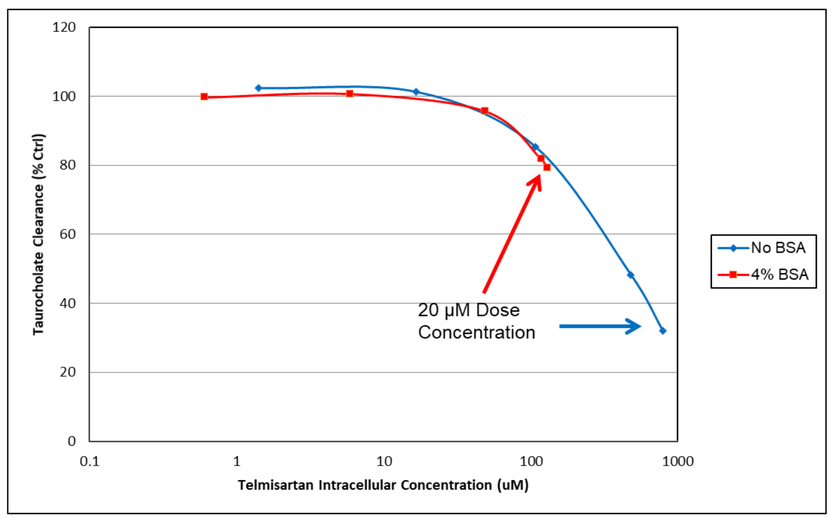

Figure 4 Inhibition of taurocholate biliary clearance as a function of the intracellular concentration of telmisartan in SCHH. Inhibition of taurocholate biliary clearance **depends only on the intracellular concentration of telmisartan**,

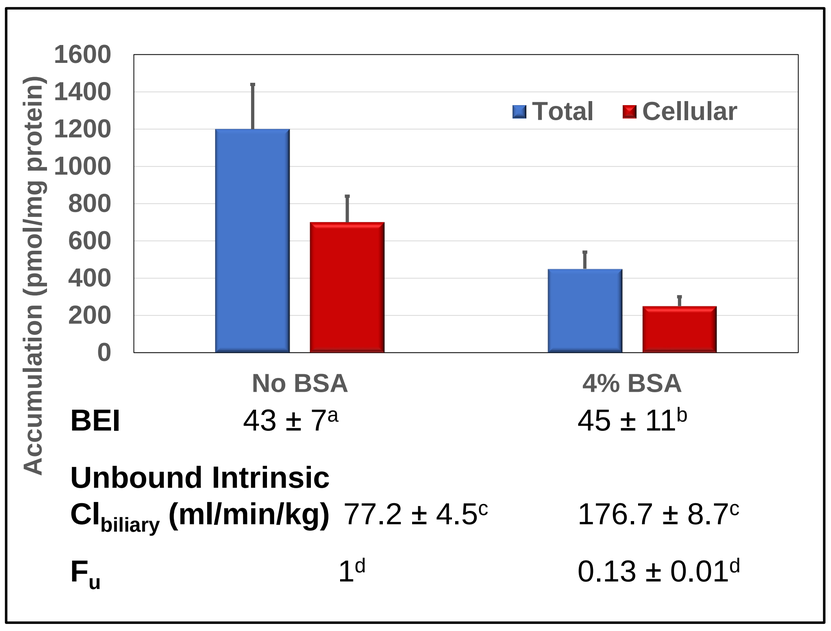

Figure 5 Rosuvastatin hepatic accumulation (Total and Cellular), biliary excretion index (BEI), unbound intrinsic $Cl_{biliary}$, and F_u in the absence or presence of 4% BSA in sandwich-cultured rat hepatocytes. n=3 livers in triplicate. Data are presented and mean ± SEM. Groups with the same letter indicate a statistically significant difference ($p<0.05$).

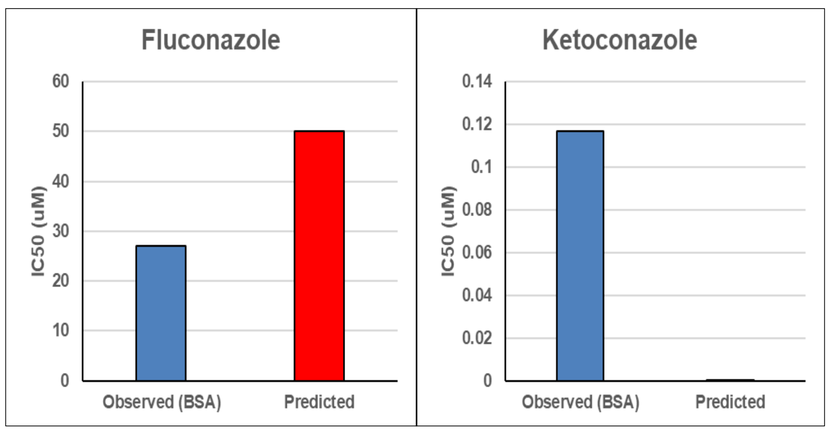

Figure 6 Effect of protein on the estimation of IC_{50} values for CYP2C9 and CYP3A4 inhibition. IC_{50} values for inhibition of CYP2C9 inhibition by fluconazole were over predicted by 2X. IC_{50} values for inhibition of CYP3A4 by ketoconazole were under predicted by 257X.

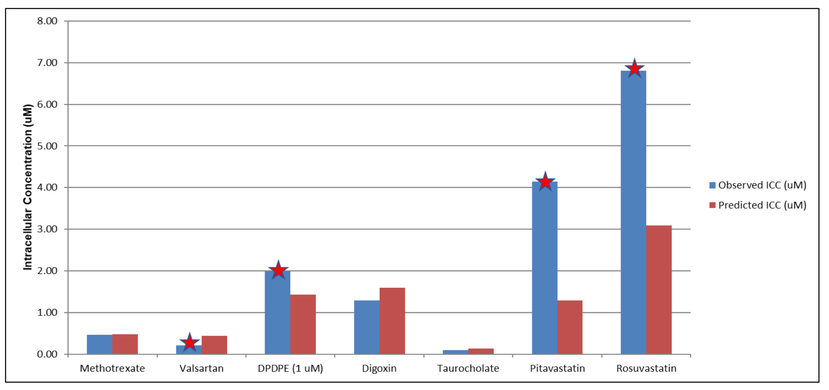

Figure 7 Comparison of intracellular concentrations (Total) for selected compounds determined from TRANSPORTER CERTIFIED™, human hepatocytes in sandwich-culture using B-CLEAR® technology, in the absence and presence of 4% BSA. The intracellular concentration (ICC) was underpredicted for pitavastatin (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.

If active transport processes are involved in hepatic uptake, the slowest process will limit the hepatic uptake.

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