Protein Binding Determination in the *In Vitro* DDI Studies

Helen L. Shen, Wenbin Liu, Julia Wang, Brad Yuska, Bruce Aungst, and Zamas Lam

QPS, LLC, 110 Executive Drive, Suite 7, Newark, DE 19702

INTRODUCTION

In vitro DDI studies are performed to investigate whether the drug candidate inhibits or induces the cytochrome p450 enzymes most commonly involved in drug metabolism. It is recommended in the EMA Guideline to use the estimated or the determined unbound drug concentration in the *in vitro* system. In the situations where it is critical to have precise unbound microsomal free fraction value (such as estimation of inhibition or induction potential not followed by a clinical study), determining the fraction experimentally is recommended. The objective of this study is to determine *in vitro* protein binding in human plasma and in the incubation medium used for inhibition and induction studies.

MATERIALS AND METHODS

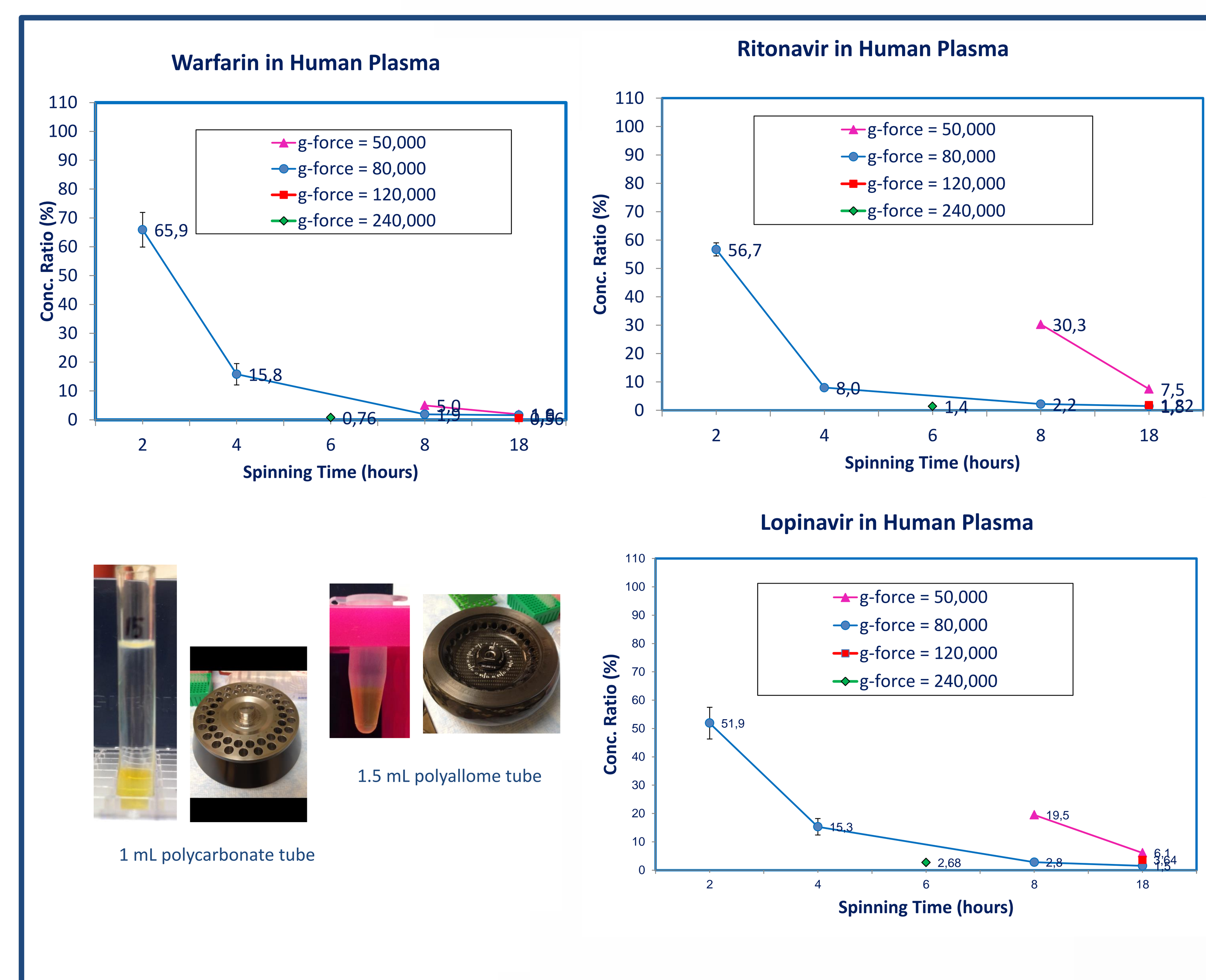
We reviewed forty compounds from existing protein binding data that involved cell culture media or microsomes.

We also investigated 4 high binding drugs in human liver microsomes (without NADPH) at 0.1 or 0.5 mg/mL microsomal protein as well as in Williams E Medium, to determine their free fractions in the CYP incubation media.

- Equilibrium dialysis (ED) was conducted with spiked plasma (2 µM, 1 mL) dialyzed against 0.133M potassium phosphate buffer (1 mL, pH 7.4) at 37°C using a Multi-Equilibrium Dialyzer™ system with a dialysis membrane of 5000 molecular weight cut off (5K MWCO).
- Ultracentrifugation (UC) was conducted with spiked matrices centrifuged at 37°C using a system consisting of a Beckman L8-70M and FiberLite Ultraspeed Rotor or Titanium Fixed-Angle Rotor (Model: F50L-24 × 1.5 or 50.4 Ti) at 50,000 to 240,000 x g.
- Time course experiments at g-force 50,000 to 240,000 were carried out with ultracentrifugation method.
- After UC, visually clear phase separation was produced and 2 aliquots (100 µL each) were removed from each UC plasma sample supernatant for analysis. ^{14}C-Warfarin, tolbutamide, ritonavir, and lopinavir were used in this study. Liquid scintillation counting or LC-MS/MS was used for bioanalysis.

PROTEIN BINDING DETERMINATION BY ULTRACENTRIFUGATION

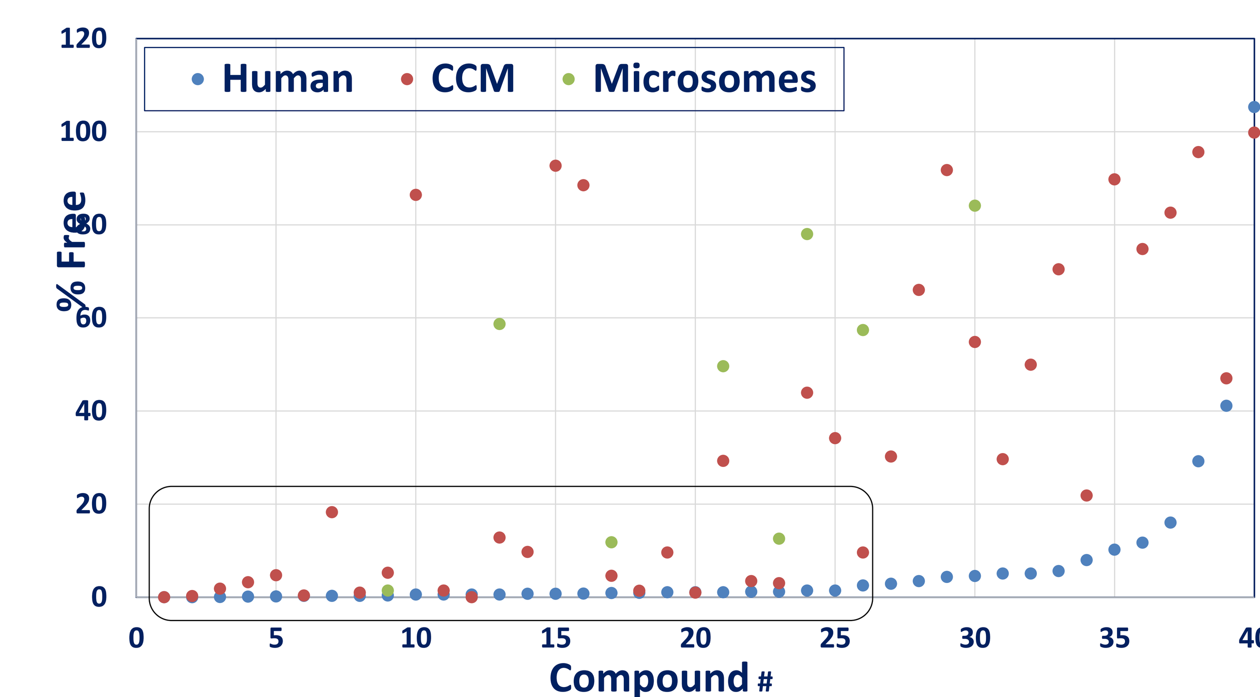
- We established ultracentrifugation method for protein binding determination at 200,000 x g and 240,000 x g previously.
- We were interested in looking into the effect of lower centrifugation g-force and the duration of centrifugation.
- Three compounds with high plasma protein binding compounds were selected: Warfarin, Ritonavir, and Lopinavir



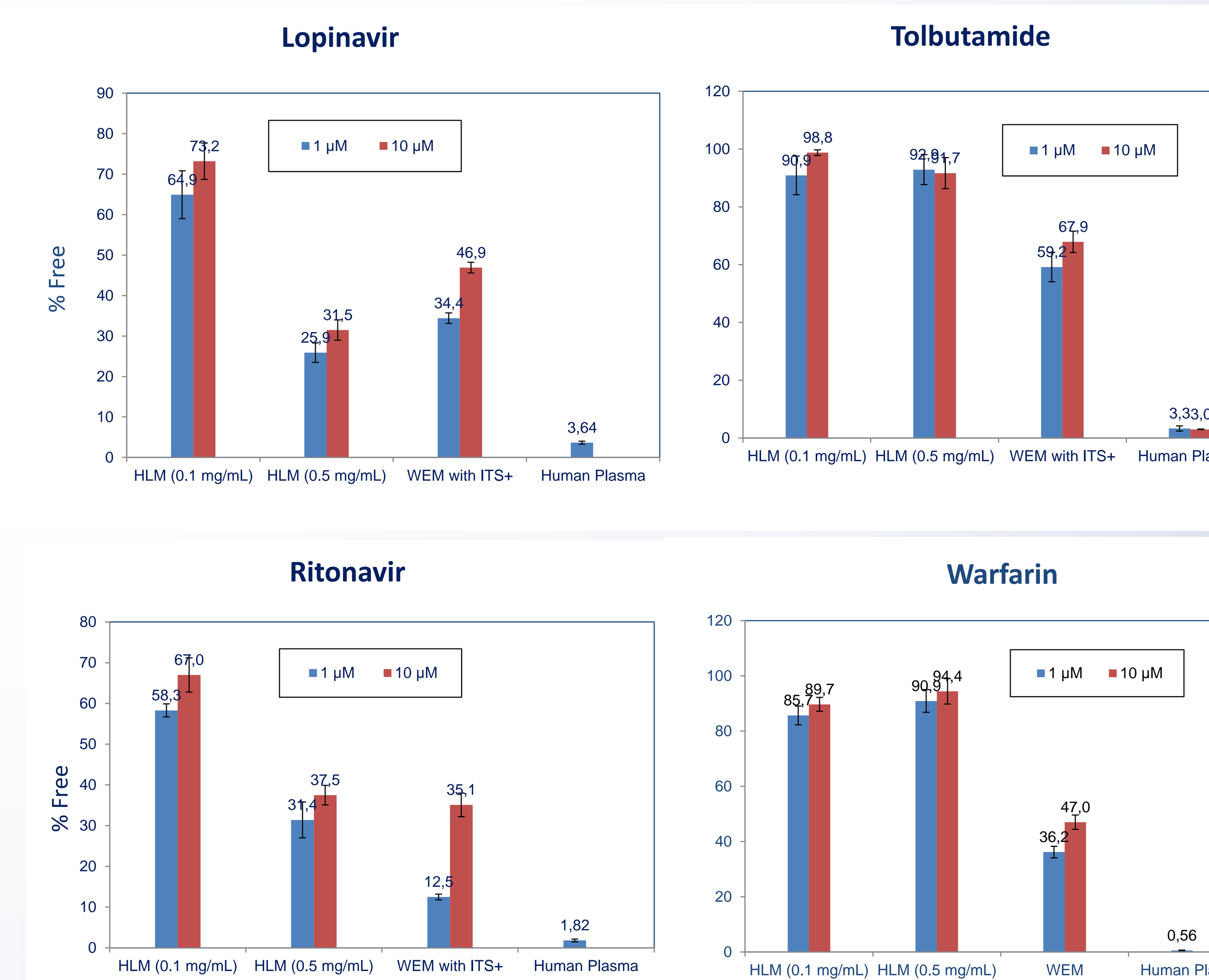
RESULTS

- Review protein binding data of 40 compounds in human plasma and cell culture media which contained 10% FBS (CCM). Eight compounds also included data in human liver microsomes (HLM)
- As expected percent free in CCM was higher than in human plasma for most compounds in this data set; however, approximately half of the compounds in this data set showed significant binding in CCM as well as in HLM.

% Free in Human Plasma And Cell Culture Medium (with 10% FBS) and Human Liver Microsomes (0.5 mg/mL)



Percent Free in Human Liver Microsomes (HLM) for Inhibition Assay (without NADPH) and WEM Medium with 1.25 mg/mL BSA for Induction Assay



CONCLUSIONS

In general, a loose correlation was observed with drugs that are highly bound to human plasma also tend to have high binding to microsomal cell culture incubation medium but to a much less extent. We have also observed many exceptions. Based on the observation of this study, experimentally determined unbound fractions would certainly be beneficial and sometimes critical in projecting *in vivo* drug-drug interaction potential.