

INTRODUCTION

Plasma protein binding is one of the ADME properties of drug candidates characterized during the drug discovery and development process. The protein binding data are generally derived from in vitro experiments during preclinical development and not until Phase II/III clinical trials are ex vivo data generated when the effects of disease state on pharmacokinetics are investigated. The objective of this study is to compare ex vivo plasma protein binding in healthy volunteers and in patients from various disease states.

MATERIALS AND METHODS

- In this study plasma samples were collected from various clinical studies and were stored at -70°C. Protein binding for more than 15 NCEs and marketed drugs as well as their metabolites was determined using equilibrium dialysis (ED), rapid equilibrium dialysis (RED), ultrafiltration (UF), or ultracentrifugation (UC). Liquid scintillation counting or LC-MS/MS was used for bioanalysis.
- The effect of renal impairment on plasma protein binding was examined for seven compounds (with eight metabolites for three of the compounds).
- Similarly, the effect of hepatic impairment on plasma protein binding was examined for five compounds
- To evaluate method variability during ex vivo protein binding determination, blank plasma spiked with the test compound was prepared for in vitro protein binding assessment in parallel. As a secondary objective, difference in protein binding determined in vitro vs. ex vivo was evaluated.
- Ultracentrifugation (UC) was conducted with plasma samples (~ 3 mL) centrifuged (200,000 x g) at 37°C for 16 hours (Beckman Coulter Optima Model: L-90K and SW60 Ti rotor)
- Equilibrium dialysis (ED): plasma samples or spiked plasma (0.7-1 mL) dialyzed against phosphate buffer (pH 7.4) at 37°C (Multi-Equilibrium Dialyzer™ system with 5K membrane.
- Rapid Equilibrium Dialysis (RED): plasma samples or spiked plasma (0.2 - 0.3 mL) dialyzed against phosphate buffer (pH 7.4) at 37°C using the Single-Use Plate with 8K membrane.
- Ultrafiltration (UF): plasma (1 mL) was equilibrated at 37°C for ~ 15 minutes before centrifuged for ~ 20 minutes using Centrifree® with 30K membrane.

RESULTS

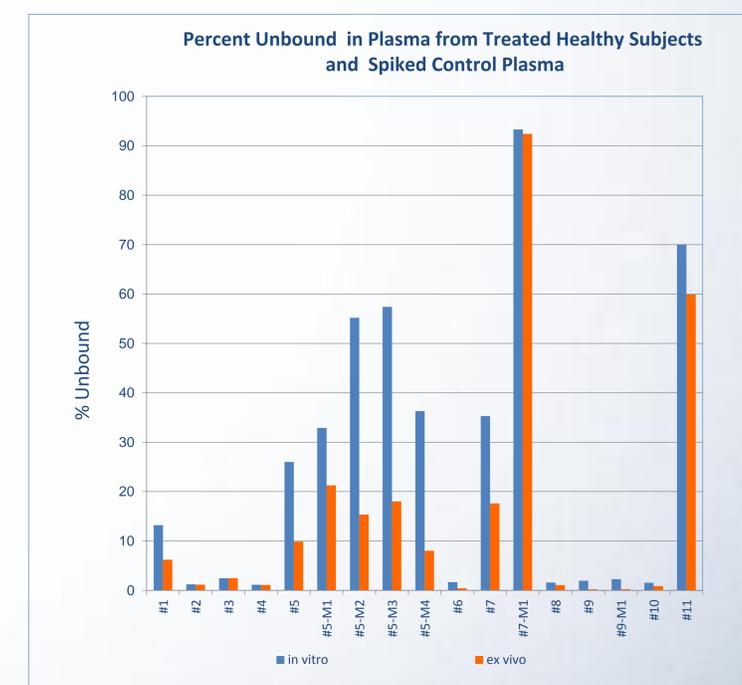
- The effect of renal impairment on plasma protein binding was examined for seven compounds (with eight metabolites for three of the compounds). No difference was observed between renal impaired and healthy subjects for the compounds or their metabolites.
- Similarly, the effect of hepatic impairment on plasma protein binding was examined for five compounds, and no significant difference was observed.
- An effect of disease on protein binding was observed for some compounds in cancer patients, most likely due to change of alpha acid glycoprotein level in these patients.

Figure 1 Effect of Renal and Hepatic Impairment on Plasma Protein Binding



- To evaluate method variability during ex vivo protein binding determination, blank plasma spiked with the test compound was prepared for in vitro protein binding assessment in parallel.
- As a secondary objective, difference in protein binding determined in vitro vs. ex vivo was evaluated.
- Of the eleven compounds investigated, we found five compounds and five metabolites derived from two of the compounds showed higher free fraction in vitro than ex vivo with two fold or more differences.

Figure 2 Percent Unbound Determined Ex vivo vs. In vitro



CONCLUSIONS

- These results demonstrate the effect of disease state on pharmacokinetics are compound dependent.
- These examples clearly demonstrated that matching healthy normal control in the same study is necessary for an appropriate comparison of plasma protein binding change due to disease state. The reason for lower binding in vitro than ex vivo in plasma from healthy subjects is not clear, as factors such as concentrations of the drug or metabolites and the chemical functionality of the metabolites cannot adequately explain the observed differences in binding.