In Vitro-In Vivo Correlation (IVIVC) for clearance estimation in earlyADME : the importance of unbound fraction assessment in plasma and microsomes

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ABSTRACT

Introduction: *In vitro* assays have been used for a long time in drug discovery in order to improve the iteration process and to allow faster screening with a larger numbers of new chemical entities while reducing the use of animals. Nevertheless, *in vitro* assays have to bring experimental data which are relevant to what will happen *in vivo* both in animals and in human. At the start of a medicinal chemistry optimization program, new chemical series may have different DMPK properties *in vivo*. An *In-Vitro/In-Vivo* Correlation (IVIVC) should be set-up to ensure that the earlyADME *in vitro* screening strategy will reflect the *in vivo* compound elimination pathway.
This investigation aimed to develop an *IVIVC* between intrinsic microsomal clearance and plasmatic clearance in mouse and to evaluate the importance of the binding to the microsomal and plasma proteins.

Method: This example of IVIVC was established on a set of 41 Inventiva compounds from the same chemical series. The *in vivo* pharmacokinetic profile was assessed at 1 mg/kg after a 3-min intravenous injection in mouse for assessment of the total plasmatic clearance. The *in vitro* metabolic stability was assessed with compounds at 1 or 0.5 µM for 30 min on 0.25 or 0.5 mg/mL of microsomes to calculate the intrinsic microsomal clearance. The assessment of the unbound fraction to the microsomes and to the plasma proteins (fup) was performed by equilibrium dialysis for 5 hours.

Results: A correlation was observed between the unbound *in vitro* intrinsic microsomal clearance and the unbound *in vivo* plasma clearance. The large range of *in vitro* and *in vivo* clearance data consolidates this correlation ($r^2 \sim 0.7$), which was not observed when the protein binding was not considered in the relationship ($r^2 < 0.5$).

Conclusion: This investigation clearly demonstrated the importance of the assessment of the protein binding in the accuracy of the IVIVC for clearance: both microsomal and plasmatic clearances should be balanced by the corresponding unbound fraction to the microsomal and plasma proteins.

OBJECTIVES

The objective was to evaluate the importance of the protein binding assessment (*in vitro* and *in vivo*) in the building of an *in vitro-in vivo* clearance correlation.

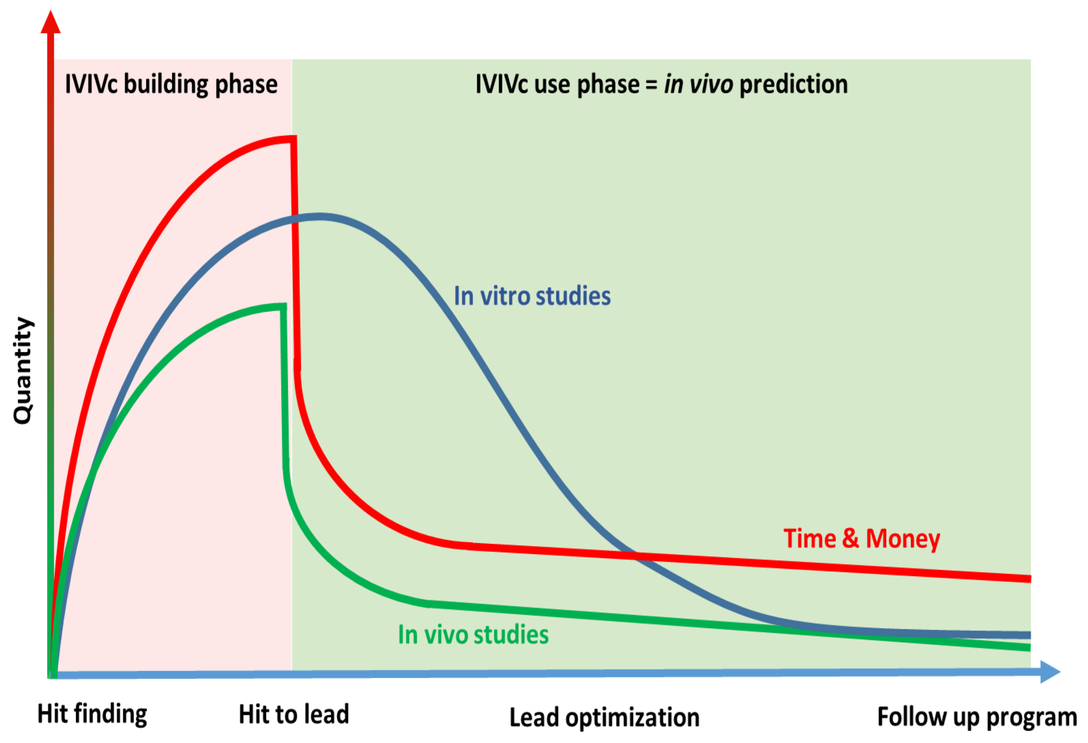


Figure: ***An In Vitro / In Vivo Correlation*** *set-up in early discovery phase* ***allows better and faster optimization*** *with less resources.*

METHOD

The set of compounds consisted of 41 Inventiva compounds.

In vivo**:** Male C57BL/6JRj mice were dosed at 1 mg/kg by a 3-min infusion at the caudal vein. Compounds were formulated in Cremophor EL 2% - 0.9%NaCl. Blood were sampled in microtubes containing 20 µL of lithium heparinate evaporated solution at 1000 IU/mL after 0.05, 0.25, 0.5, 1, 2, 4 and 7 hours post-dosing then plasma was prepared. The plasmatic clearance CL_p estimation was assessed with WinNonLin Phoenix® software, with the acceptance criteria of r^2>0.85 and AUC_{extra}% <25%.

In vitro **Microsomal Stability:** This test was performed with an automated protocol on a Caliper-Perkin Elmer robotic platform. Compounds were incubated at 37 °C at 1 µM or 0.5 µM in a 96-well plate with 0.5 mg/mL or 0.25 mg/mL of mouse liver microsomal proteins respectively and NADPH-generating system (1 mM NADP, 5 mM glucose 6-phosphate, 1 U/ml glucose 6-phosphate dehydrogenase, and 5 mM $MgCl_2$) in 100 mM Tris buffer (pH 7.4). Reactions were initiated by adding tested compound in pre-incubated reaction mixture. After 15 and 30 min, incubations were stopped by adding acetonitrile. Parent compounds were quantified for the calculation of the intrinsic clearance CL_{int} (mL/min/mg protein) and the mouse scaled liver microsomal intrinsic clearance CL_{int_scaled} (L/h/kg).

CL_{int} = slope/protein concentration ; $CL_{int\_scaled} = CL_{int}$ x (scaling factor/body weight)

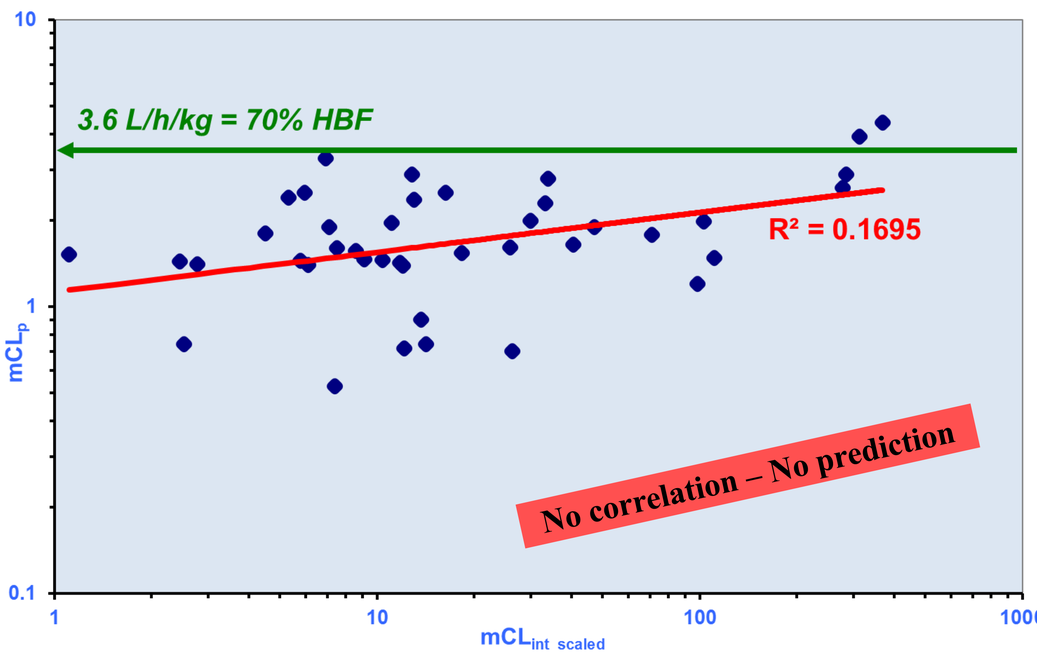
In vitro **Microsomal Protein Binding:** Compounds were incubated at 0.5 µM in 0.25 mg/mL of human liver microsomes in an HT Equilibrium Dialysis plate (HT Dialysis) for 5 hours with a semi-permeable membrane (pore size = 12-14 KDa, HT Dialysis) and a 50 mM potassium phosphate buffer as dialysate.

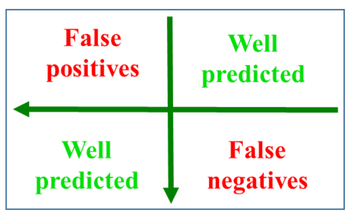
In vitro **Plasma Protein Binding:** Compounds were incubated at 1 µM in mouse plasma in an HT Equilibrium Dialysis plate (HT Dialysis) for 6 hours with a semi-permeable membrane (pore size = 12-14 KDa, HT Dialysis) and a D-PBS Dubelco buffer (Gibco) as dialysate.

The bioanalytical phase was performed using a LC-MS/MS method for all experiments.

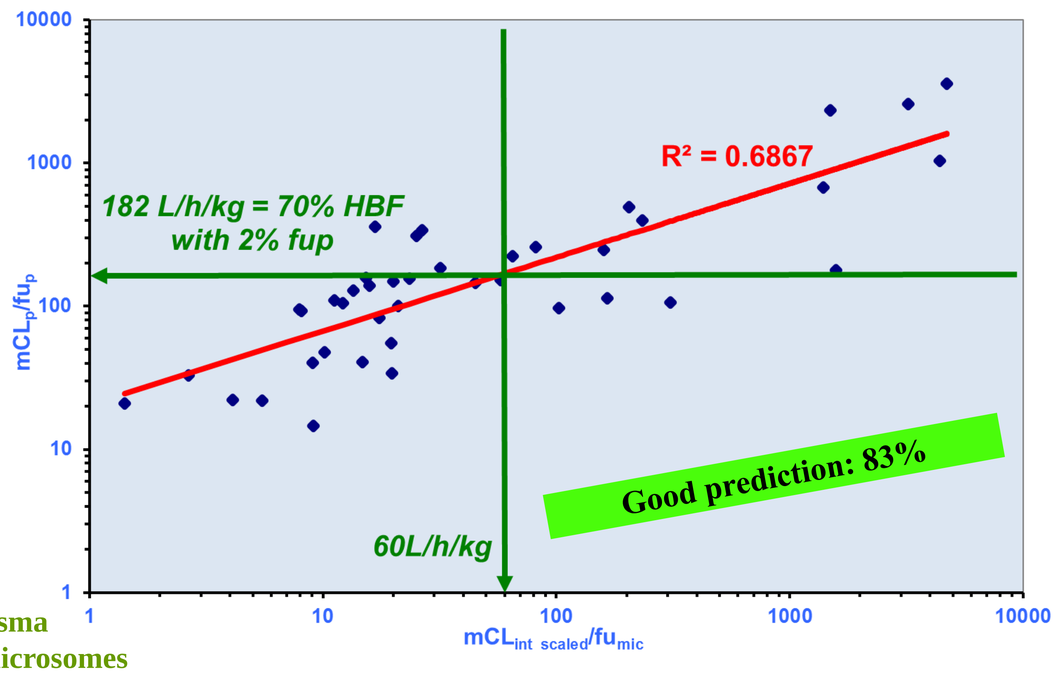
RESULTS

Graph 1: *in vivo* clearance vs *in vitro* clearance, **not considering fu_p nor fu_{mic}**





Graph 2: *in vivo* clearance vs *in vitro* clearance, **considering fu_p and fu_{mic}**



fu_p: unbound fraction in plasma
fu_{mic}: unbound fraction in microsomes

DISCUSSION

A correlation was observed between the unbound *in vitro* intrinsic microsomal clearance and the unbound *in vivo* plasma clearance. The large range of *in vitro* and *in vivo* clearance data consolidates this correlation ($r^2 \sim 0.7$), which was not observed when the protein binding was not considered in the relationship ($r^2 < 0.2$). During early ADME screening, a go/no go criteria for compound selection was established *in vivo* at 70% of the hepatic blood flow, corresponding to the *in vitro* go/no go criteria clearance at 60 L/h/kg. Based on these criteria, the observed IVIVC would allow very good prediction of the clearance parameter: > 80% good prediction ; < 7% of false negative.

This investigation clearly demonstrated the importance of the protein binding assessment in the clearance IVIVC for screening: both microsomal and plasmatic clearances should be corrected by the corresponding unbound fraction to the microsomal and plasma proteins.

A clearly established IVIVC for a new chemical series during hit to lead stage will allow to consolidate the relevancy of the *in vitro* assays, to define more precisely the *in vitro* criteria for compound progression in *in vivo* assays and to improve the optimization efficiency of hit and lead compounds.