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Perspective

Industry Perspective on Contemporary Protein-Binding Methodologies: Considerations for Regulatory Drug-Drug Interaction and Related Guidelines on Highly Bound Drugs

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ABSTRACT

Regulatory agencies have recently issued drug-drug interaction guidelines, which require determination of plasma protein binding (PPB). To err on the conservative side, the agencies recommend that a 0.01 lower limit of fraction unbound (f_u) be used for highly bound compounds (>99%), irrespective of the actual measured values. While this may avoid false negatives, the recommendation would likely result in a high rate of false positive predictions, resulting in unnecessary clinical studies and more stringent inclusion/exclusion criteria, which may add cost and time in delivery of new medicines to patients. In this perspective, we provide a review of current approaches to measure PPB, and important determinants in enabling the accuracy and precision in these measurements. The ability to measure f_u is further illustrated by a cross-company data comparison of PPB for warfarin and itraconazole, demonstrating good concordance of the measured f_u values. The data indicate that f_u values of $\leq$0.01 may be determined accurately across laboratories when appropriate methods are used. These data, along with numerous other examples presented in the literature, support the use of experimentally measured f_u values for drug-drug interaction predictions, rather than using the arbitrary cutoff value of 0.01 as recommended in current regulatory guidelines.

Abbreviations used: AUC, area under the curve; AUCR, area under the curve ratio; AAG, alpha 1-acid glycoprotein; CYP, cytochrome P450; BSA, bovine serum albumin; CRO, contract research organization; CV, coefficient of variation; DCC, dextran-coated charcoal; DDI, drug-drug interaction; DMLG, drug metabolism leadership group; DMPK, drug metabolism and pharmacokinetics; EC_{50}, half maximal effective concentration; FBS, fetal bovine serum; f_u, fraction unbound; $f_{u,inc}$, fraction unbound under incubation conditions; $f_{u,cell}$, fraction unbound in cells; HSA, human serum albumin; IQ, International Consortium for Innovation and Quality in Pharmaceutical Development; K_i, inhibitory constant; LLOQ, lower limit of quantification; LSC, liquid scintillation counting; PBS, phosphate buffer saline; PD, pharmacodynamics; PK, pharmacokinetics; PK/PD, pharmacokinetics/pharmacodynamics; PPB, plasma protein binding; TDI, time-dependent inhibition; TI, therapeutic index; RE, relative error; QC, quality control.

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Introduction

Plasma protein binding (PPB) is one of the most fundamental drug metabolism and pharmacokinetics (DMPK) parameters used to develop PK/PD relationships, predict drug-drug interactions (DDIs), and evaluate toxicity of drug candidates.[1,2] PPB is an essential property to consider when predicting the human PK of drug candidates using *in vitro* data obtained with human reagents. However, it is not a property that should be 'optimized' in most cases.[1,3] Indeed, there are many drugs on the market with very high PPB, and trends indicate that the percentage of drugs with PPB above 99% is increasing. Preclinical assessment of DDI risk is essential for drug candidates and is generally based on unbound human drug levels. However, due to the historical uncertainty of standard methodologies for measuring fraction unbound (f_u) of highly bound drugs,[4] regulatory agencies (e.g., European Medicines Agency [EMA] and U.S. Food and Drug Administration [FDA]) have considered the ability to accurately measure f_u for highly bound compounds to be an area of low confidence. As such, current DDI guidelines somewhat arbitrarily cap the lower limit of PPB f_u values at 0.01 to err on the conservative side of DDI prediction to avoid false negatives.[5,6] As the decision criteria for whether a clinical DDI study may be needed is affected by the unbound inhibitor or inducer concentration, this 0.01 f_u lower limit may result in higher *predicted* DDI risk for highly bound compounds and therefore a recommendation to conduct a clinical DDI study. In contrast, if an accurate experimentally determined f_u value could be used, the decision criteria may not have necessitated a DDI study. With roughly a third to a half of the experimental drug space exhibiting high protein binding ($\geq$99%),[4,7] using the 0.01 f_u lower bound can lead to unnecessary clinical DDI studies and more stringent inclusion/exclusion criteria for clinical study protocols, which may unnecessarily add cost and time in delivery of new medicines to patients.

Historically poor accuracy in quantifying high protein binding was influenced by methodological discordance.[4,8] Specifically, PPB studies in drug development were commonly conducted with radiolabeled drug material, with high PPB reported as equal to or greater than the radiochemical purity, because bioanalysis of low drug concentrations was technically challenging before routine implementation of modern mass spectrometric detection. Practically, radiochemical purity is generally no greater than 99%, and so this approach using radiochemical detection is inherently unable to quantify $f_u < 0.01$. Although this limitation can be overcome by combining radio-detection with chromatographic separation, this approach was rarely taken as pre-2012 regulatory recommendations focused on assessing PPB solely for reporting purposes in drug labels with key clinical DDI decisions being driven by total drug levels.[9,10] Furthermore, before miniaturization of equilibrium dialysis devices in the 1990s, this approach was often bypassed in favor of technically easier methodologies, such as ultracentrifugation and ultrafiltration. Unfortunately, these early approaches may perturb the equilibrium conditions necessary for robust measurement of low f_u values, may be confounded by extensive nonspecific binding to the apparatus, and, therefore, were generally less reliable to accurately and reproducibly determine f_u values for highly bound compounds.[11-13]

Owing to the fundamental importance of accurate PPB measurement in developing PK/PD relationships, predicting DDIs, and evaluation of toxicities,[1] variations of equilibrium dialysis with contemporary bioanalytical approaches have been successfully implemented by many pharmaceutical companies.[4,14] These approaches often originated in support of drug discovery and were later adapted for support of clinical drug development.[15-19] In light of the technological advancement and improvement of the mass spectrometric quantification of very low concentrations which enable the measurement of very low f_u values with sufficient accuracy and precision, there is a need to examine the use of an arbitrary f_u cutoff value of 0.01 (as mandated in the DDI guidances) for DDI predictions. The Drug Metabolism Leadership Group of the International Consortium for Innovation and Quality in Pharmaceutical Development formed a PPB working group with members from several pharmaceutical companies to promote in-depth scientific understanding of PPB and assess the accuracy of current approaches in measuring f_u for highly bound compounds. A survey was conducted on (1) how pharmaceutical companies measure PPB during drug development, (2) current practices on determination of f_u in *in vitro* assays both for predicting metabolic- and transporter-mediated perpetrator DDI, and (3) the specifics of f_u determination in renal and hepatic impairment clinical studies. Data for 2 highly bound compounds, warfarin and itraconazole, were compared across the companies to assess both the accuracy and variability in PPB measurement. Eleven companies participated and contributed experimental f_u measurements in the cross-company data comparison. Results from this survey reflect the current practices for measuring PPB across the industry, including the use of cross species PPB values for the assessment of safety margins between animal species and humans, as well as the measurement of protein binding in *in vitro* systems and PPB in diseased states. Furthermore, contemporary protein-binding methodologies, approaches to address the challenges associated with accurately determining f_u for highly bound compounds, and the role of accurately measured PPB in DDI translation are reviewed and discussed. Our data and analyses support the use of experimentally measured f_u values, as long as adequate methods are used, for DDI predictions for highly bound compounds, rather than using the arbitrary cutoff value of 0.01 as recommended in current regulatory guidelines.

Current PPB Practices in Pharmaceutical Companies

To understand the current practices of PPB in pharmaceutical companies, a survey was conducted on various aspects of PPB, including methodologies, acceptance criteria, data interpretation, accuracy of measurement for highly bound compounds, and PPB in hepatic and renal impairment special populations. This information was used to develop recommendations on PPB practices and provide guidance on how to use the data for DDI prediction. This section summarizes the current PPB practices based on survey results provided by the participants.

Methodologies for PPB Measurement

For PPB measurement, the details from the survey are summarized in Table 1. Both in-house and contract research organizations are used to generate PPB data within the participating companies. Despite the common approaches among the companies, differences exist on various levels. Equilibrium dialysis is the most common method used in the pharmaceutical industry for measuring PPB. It is considered the preferred method as the impact of nonspecific binding is minimized in most cases when equilibrium is achieved in the system[20,21]; however, nonspecific binding can be a confounding factor in this assay for very highly bound drugs when clinically relevant efficacious concentrations are very low (presaturation method can be used to overcome this challenge, see section on Challenges in PPB). For certain compounds, such as covalent inhibitors, ultrafiltration, or ultracentrifugation may be more suitable. The equilibrium dialysis methodology has also been modified to overcome other challenges of difficult PPB measurement for some compounds, such as those that are highly bound to proteins, have high MW, are highly lipophilic, insoluble, and have high