The Development and Validation of a Computational Model to Predict Rat Liver Microsomal Clearance

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ABSTRACT: As the cost of discovering and developing new pharmaceutically relevant compounds continues to rise, it is increasingly important to select the right molecules to prosecute very early in drug discovery. The development of high throughput *in vitro* assays of hepatic metabolic clearance has allowed for vast quantities of data generation; however, these large screens are still costly and remain dependant on animal usage. To further expand the value of these screens and ultimately aid in animal usage reduction, we have developed an *in silico* model of rat liver microsomal (RLM) clearance. This model combines a large amount of rat clearance data ($n = 27{,}697$) generated at multiple Pfizer laboratories to represent the broadest possible chemistry space. The model predicts RLM stability (with 82% accuracy and a kappa value of 0.65 for test data set) based solely on chemical structural inputs, and provides a clear assessment of confidence in the prediction. The current *in silico* model should help accelerate the drug discovery process by using confidence-based stability-driven prioritization, and reduce cost by filtering out the most unstable/undesirable molecules. The model can also increase efficiency in the evaluation of chemical series by optimizing iterative testing and promoting rational drug design. J Pharm Sci 98:2857–2867, 2009

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INTRODUCTION

While the cost of drug discovery continues to soar, new and innovative approaches to reign in cost are being explored across the industry. These new approaches range from outsourcing, to a complete re-evaluation of how drug discovery organizations approach drug discovery. Deep knowledge of biologic pathways, application of preclinical PK/

Abbreviations used: ADMET, absorption, distribution, metabolism, excretion and toxicity; RLM, rat liver microsomes; HLM, human liver microsomes; QSAR, quantitative structure–activity relationship; OOB, out-of-bag; IVIVC, *in vitro*–*in vivo* correlations.

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PD, and *in silico* approaches are all being explored as accelerators in developing disease intervention strategies. Companies that can rapidly evolve their approaches will have the greatest chance of succeeding in this increasingly competitive environment.[1,2] Patent expirations and increasing demands on the health-care industry by patients, payers, and regulators will continue to drive significant impact on both pharmaceutical and biotechnology companies for the next decade.

The development and implementation of computational models in drug discovery is one approach to mitigate the cost and improve the efficiency of drug discovery. Models have been described for many ADMET properties, such as human liver microsomes (HLM), permeability, solubility, and cytochrome P-450 (CYP) inhibition.[1,3,4] Most of these models have focused on predicting experimental endpoint values in human. While these endpoints are clearly important to researchers, they do not address the need for predictive models in nonclinical species, such as the rodent. Predictive models in rodents are important because the vast majority of drug discovery work is performed in this species. The rat is still the most commonly used animal model for evaluation of pharmacokinetics, is widely used as a non-clinical safety species, and is also used in many pharmacology and disease models. With regard to the latter, the need to bridge predictions between those in rat and those in human is becoming increasingly important as the industry focuses on translational research as a means to improve phase 2 survival. It is our aim that the development and implementation of a computational rat liver microsomal (RLM) model will aid in predicting pharmacokinetic properties in rat in order to anticipate issues with exposure in disease and pharmacology models, and as a means to ultimately support development of *in silico* PK/PD models to further improve efficiency in early drug discovery. Taken together, the cooptimization of RLM (preclinical) and HLM (clinical) should ultimately reduce the number of compounds that need to be synthesized and tested. This multiparameter optimization is being attempted across the industry with varying success.[5–7]

RLM assays are frequently used in preclinical settings to evaluate apparent intrinsic hepatic clearance in rat. Since microsomes are vesicles of the smooth endoplasmic reticulum of hepatocytes, the RLM assay measures metabolism of drugs primarily by CYP enzymes, which is the major route of drug metabolism. CYPs contribute to the clearance of roughly 75% of the top 200 prescription medications in the USA.[8]

In addition to measuring intrinsic hepatic clearance, which helps rank order compounds to derive structure–activity relationships, RLM data are also used to evaluate *in vitro–in vivo* correlations (IVIVC) in rat when compared to *in vivo* hepatic clearance data. Observation of such correlations will enhance confidence in using *in vitro* results to guide dosing in pharmacology and disease models in rats. Moreover, establishment of IVIVC in rats provides confidence in using human *in vitro* metabolism data from HLMs to predict human *in vivo* hepatic clearance.

Limited throughput and high cost of running RLM assays make *in silico* approaches a more attractive alternative. In this study, we describe the experimental data generation, data curation, model generation, and validation for predicting RLM clearance. The capability to estimate intrinsic hepatic clearance prior to compound synthesis, along with other parameter estimates can significantly influence which compound medicinal chemists synthesize next. The recovered opportunity cost from deferring synthesis of metabolically unstable compounds can be significant. Successful applications of *in silico* approaches to study ADMET properties have been frequently reported[9–11] and extensively reviewed.[12–15]

METHODS

Data Generation

Data were generated from high throughput metabolic stability assays performed at four Pfizer Research Laboratories. All laboratories used a centrally prepared batch of RLM, prepared by XenoTech (Lenexa, KS). Table 1 contains a summary of the conditions associated with each of the individual assays across the four laboratories. In brief, microsomes were thawed, resuspended in buffer, and incubations were conducted with test compounds in 96-well or 384-well plates using various automated and semiautomated methods. Samples were collected at various time points over the course of the incubation for analysis via LC/MS/MS. Half-life was calculated using standard pharmacokinetic techniques and apparent intrinsic clearance was subsequently calculated for each compound.