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PERSPECTIVE

Clinical Translation in the Virtual Liver Network

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The liver is the central detoxifying organ, continuously removing xenobiotics from the vascular system. Given its role in drug metabolism, a functional understanding of liver physiology is crucial to optimizing drug efficacy and patient safety. The Virtual Liver Network (VLN), a German national flagship research program, focuses on producing validated computer models of human liver physiology. These models are used to analyze patient-derived data and thereby gain mechanistic insights in the processes underlying drug pharmacokinetics (PK).
CPT Pharmacometrics Syst. Pharmacol. (2014) **3**, e127; doi:10.1038/psp.2014.25; published online 30 July 2014

PERSPECTIVE

Translation of knowledge generated in *in vitro* assays or in animal models to clinical trials in humans is an essential process in pharmaceutical development. As attrition rates are generally high,[1] novel, knowledge-driven concepts for clinical trial design are clearly needed. Mechanistic computational models can support the various stages in drug development by providing a platform for data integration, data analysis, and transfer of knowledge. The VLN addresses these essential steps by analyzing measured PK of a standardized cocktail of marketed drugs in both mice and humans. These data are used to quantify clearance rates in individualized physiology-based pharmacokinetic (PBPK) models,[2,3] which are used for further analyses.

VERTICAL MODEL INTEGRATION IN THE VLN

Different kinds of computational models are developed in the VLN, covering various levels of biological organization. At the cellular scale, models describe intracellular signaling, cellular metabolism, or cell-cell communication; at the organ level, transport processes are mainly considered, quantifying diffusion of proteins within the lobulus, including liver perfusion by the portal vein and the hepatic artery. Hepatic blood flow rate is of particular importance for *in vivo* simulations since it links the liver with the rest of the body. The organism level can be described by pharmacokinetic models, which may be used to translate an administered drug dose to plasma concentration levels and further on to exposure profiles within the liver. Ultimately, consideration of the rest of the body allows the quantification of vascular recirculation such that the liver can be described within an *in vivo* context.

Integrating different modeling approaches into a multiscale simulation framework enables physiological cofactors, such as patient genotype or specific liver pathologies, to be related to macroscopic observations at the whole body level. Enzymatic phenotypes can thus be translated to changes in plasma concentration levels, which means that genetic predisposition of patients can be correlated to diagnosable,

physiological markers. A mechanistic understanding of processes governing drug PK significantly supports the establishment of such quantitative correlations.

THE ROLE OF THE LIVER IN DRUG DETOXIFICATION

Detoxification in the liver is performed primarily in hepatocytes, with blood flow determining the supply to the liver, and drug-metabolizing enzymes or drug transporters controlling cellular turnover. Drug-metabolizing enzymes such as cytochrome P450 enzymes (CYPs) or UDP-glucuronosyl transferases (UGTs) are important in the biotransformation of drugs, while drug transporters facilitate the uptake (e.g., organic anion and cation transporters, organic anion–transporting peptides (OATPs)) and efflux (e.g., multidrug resistance proteins, P-glycoprotein) across membranes.[4] The coordinated action of the product of these genes at organism level supports absorption, distribution, metabolism, and excretion of foreign compounds, thus controlling drug exposure.[5] Note that PBPK models provide a mechanistic platform for integration and representation of physiological processes underlying drug detoxification within a whole-body context.[2,3]
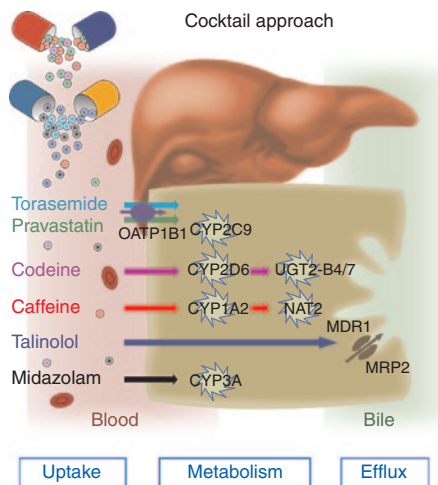
THE VIRTUAL LIVER DRUG COCKTAIL

In the VLN, hepatic capacity *in vivo* is quantified by administering a standardized cocktail of marketed drugs to probe the activity of specific drug transporters or drug-metabolizing enzymes, and pharmacokinetic absorption, distribution, metabolism, and excretion processes are analyzed with minimally invasive procedures (**Figure 1**). Total clearance of a probe drug eliminated exclusively by one enzyme and partial metabolic clearance (if several enzymes are involved) are used as metrics. Absorption and elimination rate constants are suitable pharmacokinetic parameters to characterize the function of drug transport proteins.[5,6] The simultaneous phenotyping for drug-metabolizing enzymes and drug transporters using a "cocktail" of test drugs (pharmacological phenotyping) is a well-established procedure to assess quantitatively the effect of an intervention (e.g., drug therapy,

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diet) or a condition (e.g., genetic polymorphism, disease) on absorption, distribution, metabolism, and excretion pathways during drug development. Test drugs can be selected to cover specifically the metabolic and transport pathways under investigation. Likewise, some drugs are administered orally and intravenously to separate intestinal and hepatic processes since both routes of application can be mechanistically represented within PBPK models.[2,3] The VLN cocktail consists of oral codeine (CYP2D6, UGT2B7), caffeine (CYP1A2, NAT2), talinolol (ABCB1, ABCC2) and pravastatin (OATP1B1), oral and intravenous midazolam (CYPA4),

torasemide (CYP2C9, OATP1B1) as well as intravenous metoprolol (CYP2D6) used as an alternative in a subset of subjects. Importantly, drug-drug-interactions are avoided in this cocktail since the respective compounds are metabolized or transported by nonoverlapping enzymes or transporters.

State-of-the-art high sensitivity mass spectrometry allows administration of subtherapeutic doses and requires minimal blood sample volumes (≤ 100 µl), thus minimizing clinical study-associated risks. Hence, pharmacological phenotyping becomes a minimally invasive tool for *in vivo* quantification of hepatic detoxification capacity, and can also be applied to study the influence of disease on liver function in patients.

The drug cocktail is administered to both healthy volunteers and to patients undergoing hepatic surgery, enabling the quantification of different physiological factors responsible for interindividual variability in pharmacokinetic profiles, and also permitting the investigation of how pathophysiological changes in the liver affect clearance rates. Mechanistic descriptions of the pathological modifications at the cellular level are investigated within VLN. The integration of detailed cellular level pathological processes into pharmacokinetic models at the whole-body level is only at the beginning, but it will provide important mechanistic insights for applications in systems medicine and systems pharmacology in the future.[6]

Figure 1 The Virtual Liver Drug Cocktail. A standardized drug cocktail consisting of six marketed drugs is used within the Virtual Liver Network to quantify hepatic capacity *in vivo*. The test drugs are chosen to cover specific drug-metabolizing enzymes or drug transporters. The cocktail thus allows pharmacological phenotyping in humans in a minimally invasive manner.

IMAGING LIVER BLOOD FLOW

Pharmacokinetic measurements are complemented by Doppler ultrasound and magnetic resonance imaging techniques. Both approaches are used to evaluate blood flow through major arteries and veins in the arms, legs, neck, and major organs. The value of Doppler ultrasound of hepatic blood flow may be compromised by the relatively high intra- and interobserver variability and observer dependency. Compartmental analysis
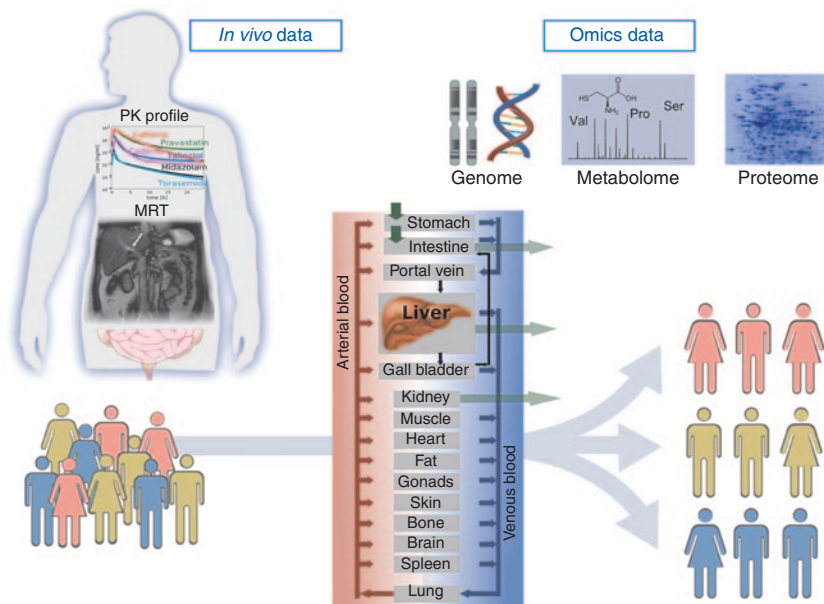
Figure 2 Patient subgroup stratification. Physiology-based pharmacokinetic modeling provides a structural template for integration of physiological *in vivo* data and omics measurements from biopsies. The individualized models allow a mechanistic explanation of the pharmacokinetics in individual patients and enable furthermore the differentiation of specific patient subgroups.

of intensity vs. time curves for MR images of the liver after injection of gadolinium chelate has been validated recently as a superior alternative to measure flow in the portal vein and the hepatic artery, proposing magnetic resonance imaging as a promising, although expensive, alternative for the noninvasive determination of hepatic function in liver disease.[7]

PHYSIOLOGY-BASED PHARMACOKINETIC MODELING

The patient-specific information generated within VLN is integrated in PBPK models, thereby mechanistically relating a patient's physiology to the emerging pharmacokinetic profile. Much physiological information quantifying, for example, blood flow rates or relative tissue-specific gene expression is explicitly provided in the basic structure of PBPK models.[2,3,8] Such parameters are generally taken from data collections, but an additional integration of specific experimental measurements in individual patients is straight forward. For example, liver volume or liver perfusion rates are structural model parameters that permit the direct integration of experimental imaging data. The same holds for the enzymatic phenotype determined from individual patient samples, which can be used to estimate changes in clearance rate constants.[2,3] Including such targeted information in a generic PBPK modeling environment enables the explanation of PK in individual patients and creates a mechanistic framework for model-based stratification of patient subgroups (**Figure 2**).

CLINICAL TRIAL DESIGN AND ANALYSIS

Planning and analysis of clinical trial data are key tasks in drug development, focusing especially on dose finding with respect to drug efficacy and patient safety.[3] However the *post hoc* identification of patient subgroups with specific response profiles is of great potential interest in optimizing therapeutic outcomes and to avoid adverse events. Using modeling together with targeted experimental data, VLN has developed a standardized workflow for risk assessment based on PBPK modeling.[3] Here, incidence rates of adverse events in specific subgroups are predicted based on carefully validated PBPK models and clinical data from dominant patient subgroups. Since this approach requires prior identification of genotype-specific differences in PK data, it is applicable at the earliest in phase III when a mechanistic PK/PD understanding for a specific drug has already been established, and a sufficient amount of patient data is available. In a complementary approach, Bayesian PBPK modeling has been used to identify pharmacokinetic phenotypes in patients where prior genotyped information was neglected or not available. For the case of pravastatin, it was shown that different phenotypes of the OATP1B1 liver transporter emerge when applying a Markov chain Monte Carlo approach to a heterogeneous cohort of patients.[9] Using this approach for clinical trial data from phase I or phase II together with mechanistic PBPK models structurally reflecting the governing absorption, distribution, metabolism, and excretion processes allows an early differentiation of specific patient subgroups. This has important implications for clinical trial design and optimization of risk–benefit ratios.

CROSS-SPECIES EXTRAPOLATION WITHIN VIRTUAL LIVER

Although the VLN drug cocktail is specifically developed for humans, it is also applied to mice for cross-species analyses. This allows us to complement human trials with invasive experiments in animal models. Likewise, bridging concepts for the transition from preclinical research to clinical trials can be evaluated by comparing PK data for the same drug cocktail in both species. This addresses a key step in drug development, involving, in particular, dose finding for first-in-human trials where patient safety has to be guaranteed.

OUTLOOK

The US Food and Drug Administration has recently accepted PBPK-based predictions for drug-drug interactions without clinical validation as an element in the drug label for ibrutinib,[10] exemplifying how computational simulations can be used as a valuable tool to complement experimental data. In general, computational models may be used as a structural platform for the representation, integration, and analysis of experimental information. The specific data generated within VLN allow a systematic physiological characterization of the liver up to the level of individual patients. Concepts for patient subgroup stratification have already been developed within VLN. The integration of patient-specific physiology into PBPK models provides the next important step toward the design of individualized therapeutic strategies taking into account a patient's phenotype. The integration of such data within a comprehensive modeling framework significantly contributes to a truly mechanistic understanding of processes governing PK. Systems pharmacology approaches that simultaneously consider the organism level and the cellular scale will support the development of new drugs by optimizing, for example, risk–benefit ratios based on computational models and the integration of targeted physiological information.

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