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Review

Sandwich-Cultured Hepatocytes as a Tool to Study Drug Disposition and Drug-Induced Liver Injury

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ABSTRACT

Sandwich-cultured hepatocytes (SCH) are metabolically competent and have proper localization of basolateral and canalicular transporters with functional bile networks. Therefore, this cellular model is a unique tool that can be used to estimate biliary excretion of compounds. SCH have been used widely to assess hepatobiliary disposition of endogenous and exogenous compounds and metabolites. Mechanistic modeling based on SCH data enables estimation of metabolic and transporter-mediated clearances, which can be used to construct physiologically based pharmacokinetic models for prediction of drug disposition and drug-drug interactions in humans. In addition to pharmacokinetic studies, SCH also have been used to study cytotoxicity and perturbation of biological processes by drugs and hepatically generated metabolites. Human SCH can provide mechanistic insights underlying clinical drug-induced liver injury (DILI). In addition, data generated in SCH can be integrated into systems pharmacology models to predict potential DILI in humans. In this review, applications of SCH in studying hepatobiliary drug disposition and bile acid-mediated DILI are discussed. An example is presented to show how data generated in the SCH model were used to establish a quantitative relationship between intracellular bile acids and cytotoxicity, and how this information was incorporated into a systems pharmacology model for DILI prediction.

Introduction

The liver is one of the major organs responsible for the metabolism and excretion of endogenous and exogenous molecules. Among many *in vitro* and *in vivo* model systems, primary hepatocytes remain the gold standard to assess hepatic drug metabolism and transport. Hepatocytes can be isolated from the species of interest, including humans, to address species differences in hepatic disposition of drugs. Primary hepatocytes express multiple metabolic enzymes and transporters, enabling assessment of overall hepatobiliary drug disposition. However, hepatocytes in suspension or under conventional culture conditions quickly lose cell polarity and viability, which limits their utility.[1,2] Culturing hepatocytes between 2 layers of gelled collagen (sandwich configuration) improves morphology and viability of hepatocytes and maintains function for longer periods in culture.[3,4] In addition, sandwich-cultured hepatocytes (SCH) regain polarity, allowing proper localization of basolateral and canalicular transporters as well as formation of functional bile networks (Fig. 1).[5,6]

When properly cultured, the expression and function of basolateral uptake transporters including sodium taurocholate cotransporting polypeptide (NTCP) and organic anion-transporting polypeptides (OATPs) are maintained over time in human SCH,[7-9] whereas downregulation of NTCP and OATP has been reported for rat SCH.[6,7] On isolation, hepatocytes lose biliary excretory function

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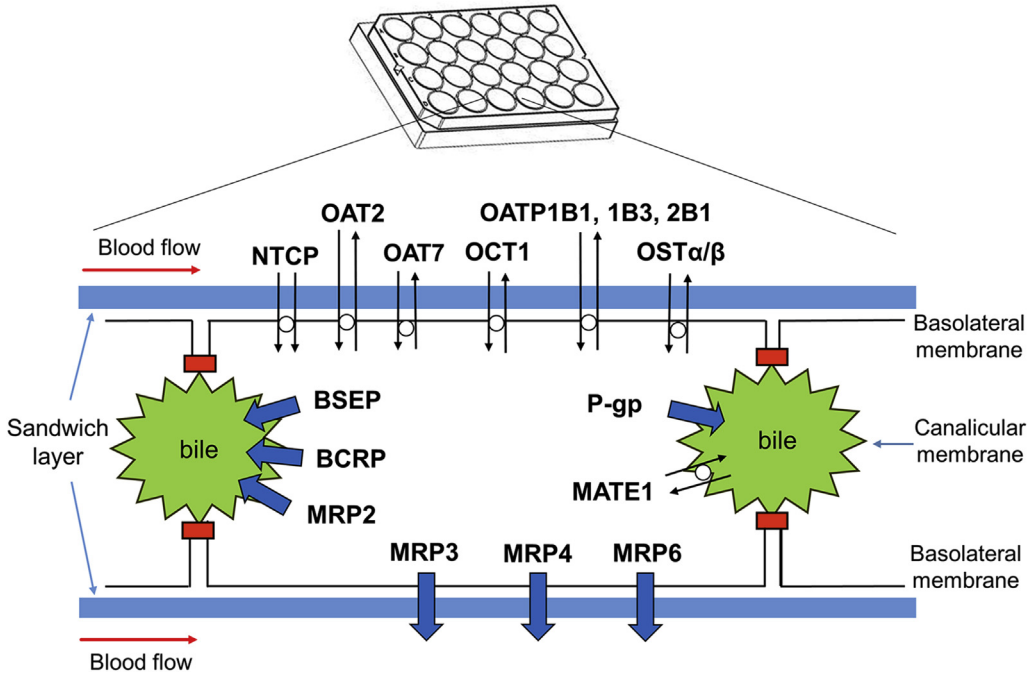

Figure 1. Scheme illustrating the polarized expression of transporters in human SCH. Three adjacent hepatocytes with interconnecting canalicular spaces sealed by tight junctions (red rectangles) are shown. Important ATP-binding cassette transport proteins are depicted by blue solid arrows denoting the direction of transport. Solute carrier transporters are depicted with black double arrows. Uptake transporters in the basolateral (sinusoidal) membrane include NTCP; organic anion transporter 2 (OAT2) and OAT7; organic cation transporter 1 (OCT1); and OATP1B1, OATP1B3, and OATP2B1. The heteromeric organic solute transporter (OSTα/β) is also depicted on the basolateral membrane. Efflux transporters expressed in the hepatocyte basolateral membrane include MRP3, MRP4, and MRP6. Canalicular (apical) efflux pumps include MRP2, BCRP, BSEP, MDR1 P-gp, and multidrug and toxin extrusion protein 1 (MATE1).

because of internalization of canalicular efflux transporters.[10] However, canalicular transport proteins (e.g., bile salt export pump [BSEP/Bsep], P-glycoprotein [P-gp], breast cancer resistance protein [BCRP/Bcrp], and multidrug resistance–associated protein [MRP/Mrp] 2) properly localize over time and regain excretory function in human and rat SCH.[5,6] A schematic representation of hepatic transporters in SCH is shown in Figure 1. Phase I (i.e., cytochrome P450 [CYP]) and phase II [e.g., UDP-glucuronosyltransferase, sulfotransferase]) metabolizing enzymes also are expressed in SCH, although some enzymes exhibit decreased expression and function over time in SCH compared with freshly isolated hepatocytes, depending on the culture conditions and medium composition.[11-13] The influence of culture conditions and culture time on the expression and/or function of enzymes and transporters in the SCH system has been reviewed in detail elsewhere and is not the main focus of this article.[1,14,15]

SCH provide a unique tool to estimate biliary excretion of compounds. Substances excreted into bile and accumulated in the canalicular network can be quantified by modulating tight junctions using buffer with and without calcium.[16,17] Therefore, SCH have been used widely to assess hepatobiliary disposition of drugs and metabolites and potential drug-drug interactions (DDIs). Transcriptional and post-translational regulatory machinery are well maintained in SCH, which makes it a suitable model for studying induction and feedback regulation of enzymes and transporters in response to compounds or other interventions.[4,18] SCH that express functional metabolic enzymes also have been used to study the pharmacology and toxicology of drugs and hepatically generated metabolites. Because of the essential role of the liver in drug elimination, it is often a target organ of drug-induced toxicity. SCH have been used in the assessment of direct cytotoxicity and in mechanistic studies to determine perturbations of biological processes and to better understand underlying mechanisms of drug-induced liver injury (DILI).

In this article, applications of SCH in studying hepatobiliary drug disposition and DILI are reviewed. First, use of the SCH system to characterize hepatic drug metabolism and transport is discussed. Predictions of *in vivo* drug disposition and clinical DDIs using SCH data combined with mechanistic and/or physiologically based pharmacokinetic (PBPK) modeling also are reviewed. The use of SCH to study mechanisms underlying DILI is discussed with a focus on bile acid-drug interactions with hepatic transporters. An example of how SCH data can be used to develop a systems pharmacology model of DILI to predict clinical hepatotoxicity is introduced.

Use of SCH in Studying Drug Disposition

During drug discovery and development, assessment of metabolism and transport of new chemical entities is important to predict clinical exposure and potential DDIs. In this section, the use of SCH to study drug disposition is reviewed with a focus on metabolism and hepatobiliary transport of compounds and DDIs as well as enzyme-transporter interplay. This section also highlights the estimation of enzyme- and transporter-mediated *in vitro* intrinsic clearance values using mechanistic pharmacokinetic modeling in addition to empirical methods. *In vitro–in vivo* extrapolation of intrinsic clearance values and the incorporation of these values into PBPK modeling are discussed.

Metabolism

SCH have been used to study drug disposition, including hepatic uptake, metabolism, and biliary excretion. Although human liver microsomes (HLMs), S9 subcellular fractions, and primary hepatocytes cultured in monolayers (without extracellular matrix overlay) are commonly used in metabolism studies of drugs and endogenous substrates, SCH are often used when extended incubation times (>24 h) are needed, after which the viability and function of primary