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INTRAHEPATIC SAMPLING FOR THE ELUCIDATION OF ANTIVIRAL CLINICAL PHARMACOLOGY

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Abstract

While the importance of the liver in clinical pharmacology is widely recognized, little is known in humans concerning its function *in vivo* at the hepatocyte level and how pharmacological functions are altered in the setting of advanced liver disease. Several recent proof-of-principle studies with first-generation DAAs have demonstrated the feasibility of serial liver sampling for pharmacological studies. These studies have begun to describe the liver-to-plasma concentration and how this ratio are altered in the setting of advanced liver disease. These data are particularly relevant to individuals with substance use disorders since many have advanced liver disease as a consequence of longstanding viral hepatitis infection or continued use of hepatotoxins such as alcohol. Future research should attempt to develop standardized and reproducible methods to assess liver drug concentration, complex drug interactions and pharmacogenomics in humans to permit an elucidation of the clinical pharmacology within the liver.

Keywords

Liver drug concentration; hepatocyte; liver drug metabolism; pharmacokinetics; pharmacodynamicsf

INTRODUCTION

The liver is tremendously important from a pharmacokinetic perspective by playing a predominant role in drug metabolism and elimination. Hepatic clearance of a drug is influenced by the inherent ability of hepatocytes to remove drug (i.e. intrinsic clearance), the flow of blood to the liver, and the relationship of drug binding to plasma proteins.[1,2] A firm foundation of how liver dysfunction (e.g. cirrhosis) changes each of these parameters and the

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resulting effect on *plasma* pharmacokinetics was established by Wilkinson et al.[1,3] However, a paucity of data exists on the effect of liver dysfunction on human *intrahepatic* pharmacokinetics. The evaluation of intrahepatic pharmacokinetics, as well as pharmacokinetics at the cellular (i.e. hepatocyte) level, could be important for maximizing the efficacy and minimizing toxicity of drugs that undergo significant hepatic clearance. Thus, significant gaps exist concerning the understanding of liver function and intrahepatic pharmacology.

To begin to address these gaps, several studies of human liver pharmacology have been conducted in patients with hepatitis C virus (HCV) infection.[4–6] HCV replicates principally in hepatocytes where it establishes a cycle of inflammation and fibrosis that can ultimately culminate in end stage liver disease, cirrhosis and hepatocellular carcinoma.[7] HCV is very transmissible in blood, resulting in high prevalence among injection drug users, many of whom have advanced liver disease based upon long duration of infection. While interferon was previously the cornerstone of HCV therapeutics, current regimens consist entirely of direct acting antivirals (DAAs) that target the intracellular viral lifecycle resulting in improved efficacy.[8] DAA concentration within an infected cell is likely an important determinant of efficacy, treatment failure, development of viral resistance, and drug toxicity, as has been observed concerning the intracellular concentration of other antivirals.[9–11]

Intrahepatic drug concentrations, however, have very rarely been measured. Since unbound systemic drug concentrations are assumed to equate with those in liver, plasma drug concentrations are considered surrogates for liver concentrations.[9,12] This relationship, however, likely varies by DAA and patient population, as genetics and disease states can modulate intracellular transporters and metabolizing enzymes, thereby affecting intracellular drug concentration.[13] For example, functional single-nucleotide polymorphisms of the organic anion transporter polypeptide (OATP)1B1 are associated with a decrease in the transport function of HIV-1 protease inhibitors and ritonavir.[14,15] To gain an understanding of liver-plasma drug concentration relationships and factors that influence these relationships, direct hepatocyte drug concentration measurements are needed. An enhanced understanding of these relationships could improve dose selection and could guide dose modifications, particularly in the setting of liver dysfunction. As discussed below, several completed studies have begun to discern these relationships.

FEASABILITY OF ASSESSING INTRAHEPATIC DRUG CONCENTATION

Studies utilizing human liver tissue collected by either fine needle aspiration (FNA) or core needle biopsy (CNB) have measured first-generation DAA concentrations (Table 1). In a study of liver pharmacokinetics and pharmacodynamics, 15 genotype 1a/b chronic HCV-infected patients received telaprevir, pegylated-interferon-alpha-2a, and ribavirin with serial FNAs.[4] Interestingly, liver telaprevir concentrations were significantly lower compared to plasma, with median liver-to-plasma ratios ranging from 0.47–0.72. In rats and mice, liver telaprevir concentrations were 35 times higher- or 5.7 – 16 times higher, respectively, relative to plasma.[16,17]

Transporters often mediate unbound drug and metabolite influx into hepatocytes or efflux into sinusoidal blood or bile (Table 2).[13] Since tissue distribution and relative abundance of transporter proteins differ substantially between species, the relevance of some preclinical models to humans may be questioned.[18] For example, the mRNA expression levels of *MDR1* (human)/*Mdr1* (animal) genes, encoding for the efflux transporter P-glycoprotein (P-gp), varies substantially not only between species, but between different organs in the same species. P-gp mRNA expression levels are increased in human liver and kidney compared to rats, while in the gastrointestinal tract the inverse occurs, expression levels are lower in humans than rats.[19] Similarly, while present in humans, OATP1B1 is absent altogether in rodents and dogs.[18] Telaprevir is an inhibitor and substrate of P-gp and an inhibitor of OATP1B1 and OATP2B1 (Table 2).[20–22] Thus, species-related differences in the expression and distribution of P-gp and OATPs may partially explain the discrepant results comparing telaprevir studies in humans with those performed pre-clinically. Consequently, an incomplete understanding of protein abundance in similar organs between species or in organs of the same species complicates translation of findings from preclinical models to humans. To address these data gaps, proteomic screens for determination of drug transporter expression and localization should be pursued. As preclinical models are used extensively in the evaluation of investigational agents, it is important to consider inter- and intra-species differences as they relate to metabolism and transporter profiles of the drug under investigation.

In another DAA study of intrahepatic and plasma drug concentrations, vaniprevir concentrations were assessed in three adult chronically HCV-infected males (Table 1).[5] Different than telaprevir, vaniprevir liver-to-plasma concentration ratios ranged from ~70 to 280, indicating substantially higher liver concentrations compared to plasma. Interestingly, one patient with advanced fibrosis had the highest liver-to-plasma ratio. Although limited to one patient, the effect of advanced fibrosis on liver drug concentrations has not been noted previously and might influence intrahepatic pharmacokinetics of medications with substantial liver metabolism. For example, decreased hepatic blood flow as a result of portal hypertension, reduced cytochrome P450 isoenzyme activity, and changes in the expression of efflux transporters, have been associated with chronic HCV infection and advanced fibrosis.[2,13,23] These factors, for example, could account for increased plasma grazoprevir concentrations observed in cirrhotic patients.[24,25]

MEASUREMENT OF INTRAHEPATIC VIRAL KINETICS

To date, the clearance rate of HCV-infected hepatocytes with combination DAA regimens has not been assessed.[26] In the first study to evaluate DAA treatment-induced intrahepatic HCV RNA decline, hepatic HCV RNA declined more slowly compared to plasma between baseline and day 4, while decline rates were equivalent between days 4 to 15, and virus was undetectable by week 8 in all samples.[4] Viral kinetic modeling suggested that liver HCV RNA decay is markedly delayed compared to plasma in telaprevir-treated patients.

Telaprevir resistant mutations were also assessed.[4] Pre-treatment, only wild-type virus was detected in plasma and liver. Post-treatment initiation, telaprevir-resistant variants in plasma were observed in 7 patients while only wild-type virus was detected in liver at any time

point. While differences in resistant isolates may exist between body compartments, two important study limitations require consideration: 1) only 8 of 15 patients underwent a week 8 FNA procedure and, 2) there was potential liver sampling bias. Consequently, the FNA procedure may not have sampled liver regions in which telaprevir-resistant variants were located.

MEASUREMENT OF INTRAHEPATIC GENE EXPRESSION

Liver gene expression in FNA or CNB samples has been evaluated in several studies.[27–36] Many older studies documented an association between viral nonresponse and increased expression of hepatic and peripheral interferon-associated genes, especially interferon-gamma inducible protein-10 (IP-10; also known as CXCL10 [C-X-C motif chemokine 10]), in HCV-infected patients on interferon and ribavirin.[27–30] The addition of telaprevir to pegylated-interferon/ribavirin changed this relationship; baseline peripheral and intrahepatic IP-10 levels (collected by FNA) were not predictive of viral response.[31]

Liver gene expression in samples collected by FNA has also identified liver-enriched genes, in both humans and dogs.[37] The FNA procedure has also been used to assess changes in fibrosis-related gene expression levels in a trial of the investigational antifibrotic agent, simtuzumab.[32] These studies illustrate the feasibility of gene expression analysis in samples collected by FNA, procedures that could be employed to evaluate changes in intrahepatic gene and protein expression induced by illicit drug use or agents used in substance use disorder treatment.

CURRENT LIMITATIONS AND FUTURE DIRECTIONS FOR LIVER SAMPLING

Although serial liver sampling is feasible, limitations include potential blood contamination and measurement bias due to small sample quantities. Liver sampling via an ultrasound guided probe appears to reduce the degree of blood contamination. Additionally, quantitation of liver-enriched gene mRNA expression in FNA samples could be used to standardize the amount of liver tissue contained within serially collected samples.[37] Several of the same genes were also enriched in dog liver, illustrating potential feasibility of the procedure across animal species.

An additional oft cited concern is the invasiveness of the liver sampling procedure as a potential deterrent to subject participation in trials utilizing the procedure. In reality, this has not been the case, in which no study participant has discontinued participation due to the liver sampling procedure. Rather, the vast majority of subjects have opted to undergo additional optional procedures as procedure-related adverse events have been minimal (Table 1).

Several experimental design issues, however, merit further investigation. First, the number of analyses that can be performed on a single sample is limited by the small sample quantity. As progressively smaller quantities of sample are required for laboratory assays, it is likely that this situation will continue to improve going forward. Second, the generalizability of results to the entire liver of those obtained from sampling one region requires further assessment. Hepatocyte drug disposition may vary according to portal and venous blood

flow rates as well as regional differences in metabolism and biliary excretion.[1,2,3,9] Studies should therefore readdress the relationship between intrahepatic drug concentrations, hepatic blood flow, and the abundance and regional expression levels of hepatic transporters and metabolizing enzymes using modern, sophisticated techniques (Table 3). For instance, fibrogenesis ultimately culminating in cirrhosis and end-stage liver disease results in collagen replacement of the normal hepatic parenchyma. Given the dynamic nature of liver remodeling during fibrogenic processes, hepatic drug concentration measurements at the hepatocyte level might be more precise and informative than classic bulk tissue approaches.

CONCLUSIONS

Although the liver is fundamental to clinical pharmacology, significant gaps exist in our understanding of liver physiology as it relates to human drug metabolism and biliary excretion. Development of standardized and reproducible methods to assess liver drug concentration in humans is a research priority to enhance our understanding of the relationship between plasma and liver drug concentrations. Medication doses selected based upon intrahepatic drug concentrations could lead to more accurate dose determinations, especially in cases of medications that have small therapeutic-to-toxicity ratios and where the liver is the site of intended pharmacologic effect. Such data could also guide dose adjustment recommendations in the setting of profound liver dysfunction, such as in individuals with decompensated cirrhosis. The alterations in liver pharmacology that occur in the setting of advanced liver disease are also significantly underappreciated. These data are of tremendous relevance to those with a history of substance use disorders, many of whom are co-infected with HIV and viral hepatitis. The demonstration of the feasibility of serial liver sampling for measurement of liver drug concentrations indicates that tools currently exist to significantly improve our understanding of the liver's role in clinical pharmacology.

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Table 1

Select Studies of Liver Sampling for Understanding Intrahepatic Pharmacology

Study	Study Participants	Treatment	Hepatic Sampling Technique	Sampling Analysis
Talal et al. 2014[4]	15 genotype 1a/b chronic HCV-infected patients; cirrhotics excluded	TVR 750 mg every 8 hours, PEG-IFN and RBV for 12 weeks followed by an additional 12 of weeks of PEG-IFN and RBV	Liver FNAs were performed at baseline prior to treatment initiation, at 10 hours after treatment initiation, on days 4 and 15, and in most subjects at week 8	Intrahepatic and plasma TVR concentrations, HCV RNA, TVR resistance, viral clonal sequence analysis and microarray analysis of liver genes
Zeremski et al. 2015[31]				Intrahepatic and peripheral CXCL10 (IP-10) protein levels
Wright et al. 2015[5]	3 genotype 1 chronic HCV-infected non-responders to PEG-IFN and RBV; cirrhosis excluded	VNV 600 mg twice daily on days 1 – 3 and VNV 600 mg single-dose on day 4. After a minimum of 30 days washout, patients received VNV 300 mg twice daily on days 1 – 3 and 300 mg single-dose on day 4.	On study day 4 of each period, CNBs were performed 6 hours post-dose in one patient and 12 hours post-dose in the other two patients for measurement of intrahepatic VNV concentrations	Intrahepatic and plasma VNV concentrations
Lejnine et al. 2015[37]	17 genotype 1 chronic HCV-infected patients with mild/ moderate to advanced fibrosis; cirrhotics excluded.	None	FNAs and CNBs were performed at a single visit, 7 days later an additional FNA procedure was performed	Gene expression profiling via microarray and pathway analyses
	4 separate dog experiments		FNA and liver punches	Gene expression profiling via microarray for cross-species validation of human methods
Talal et al. 2012[4]	20 patients with chronic liver disease and stage 1–3 fibrosis	Simtuzumab three infusions at 10 mg/kg or 3 mg/kg 2 weeks apart; twelve patients received an additional 24 weeks of dosing (700 mg every 2 weeks)	Liver FNAs performed prior to the first simtuzumab infusion (at week 4) and 2 weeks after the final infusion (week 10)	Intrahepatic mRNA for microarray analysis of genes associated with liver fibrosis

CNB = core needle biopsy; CXCL10 = C-X-C motif chemokine 10; FNA = fine needle aspiration; HCV = hepatitis C virus; IP-10 = interferon-gamma inducible protein-10; PEG-IFN = pegylated-interferon; ribavirin = RBV; TVR = telaprevir; VNV = vaniprevir

Table 2

HCV Direct Acting Antivirals as Substrates and/or Inhibitors with Select Human Hepatocyte Drug Transporters

Drug Transporters		HCV Direct Acting Antivirals (References)										
		BOC (38–41)	**DCV (42,43)**	**DSV (44–46)**	**EBR (47)**	**GZR (47–49)**	**LDV (44)**	**OBV (44,45)**	**PTV (44,45)**	**SMV (42)**	**SOF (50)**	**TVR (20–22)**
Basolateral Influx Transporters (sinusoidal blood → intrahepatic)	OATP1B1	I	I	-	-	S	-	-	S, I	S, I	-	I
	OAT1PB3	-	I	-	-	S	-	-	S, I	S, I	-	
	OATP2B1	-	-	-	-	-	-	-	I	S	-	I
Canalicular Efflux Transporters (intrahepatic → bile canaliculus)	MRP2	-	-	-	-	-	-	-	-	S, I	-	-
	BCRP	-	I	S, I	I	I	S, I	S	S, I	S	S	-
	P-gp	S, I	S, I	S, I	S	S	S, I	S	S	S, I	S	S, I
	BSEP	-	-	-	-	-	-	-	-	I	-	-

BOC = boceprevir; BRCP = Breast Cancer Resistance Protein; BSEP = Bile Salt Export Pump; DCV = daclatasvir; DSV = dasabuvir; EBR = elbasvir; GZR = grazoprevir; inhibitors = I; LDV = ledipasvir; MRP = Multidrug Resistance-associated Protein; OATP = Organic Anion Transporting Polypeptide; OBV = ombitasvir; P-gp = P-glycoprotein; PTV = paritaprevir; substrate = S; SMV = simeprevir; SOF = sofosbuvir; TVR = telaprevir

Table 3

Recommended Research Priorities for Intrahepatic Pharmacology

Preclinical Studies
• To further evaluate the relationship between preclinical models and human liver with regard to drug transporters and metabolizing enzymes. • To develop functional in vitro assays utilizing human cells for the assessment of DAAs (and other agents) and bilirubin metabolism.
Translational Studies
• To evaluate liver metabolizing enzyme and transporter gene and protein expression levels and their distribution in normal liver. • To develop accurate and reproducible methods to quantify intrahepatic drug concentrations in the whole liver and within various liver sub-compartments or cell types.
Clinical Studies
• To develop and to compare drug dosing regimens based upon intrahepatic and plasma drug concentration ratios among different patient populations. • To evaluate changes in liver metabolizing enzyme and transporter gene and protein expression levels in varying stages of fibrosis.