NON-CLINICAL STUDIES TO ASSESS THE POTENTIAL FOR DRUG-DRUG INTERACTIONS OF ACH-1625, A CLINICALLY EFFECTIVE HCV PROTEASE INHIBITOR

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ABSTRACT 1892

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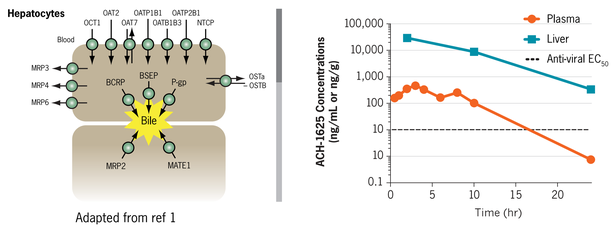
Background

ACH-1625 is a small molecule inhibitor of the HCV NS3 protease. In a phase I study, ACH-1625 was safe and well-tolerated at doses up to 2000 mg / day for 4.5-days duration. In addition, administration of ACH-1625 to Hepatitis C patients at doses of 200, 500 and 600 BID for 4.5 days or 600 mg QD for 5 days, resulted in a 3.63, 4.25, 3.94 and 3.81 $\log_{10}$ mean maximum drop of plasma HCV RNA, respectively. Suppression of viral load was maintained up to day 12. Human pharmacokinetics show that the drug is rapidly absorbed and exhibits a terminal half-life of 16 hr such that by day 12 post-dose plasma concentrations were below the anti-viral EC_{50} (11 nM).

We have previously shown hepatoselective distribution of ACH-1625 in the rat (Figure 1,right) and substrate interaction for at least 3 members of the human OATP family. Interaction with membrane transport proteins liver (Figure 1, left) can significantly effect the disposition and pharmacokinetics of many drugs [1]. In this work we explore the hepatic disposition in greater detail. In the rat, we explore the linearity of hepatic extraction and the influence of a co-administered OATP substrate, atorvastatin. We next describe the excretion characteristics of ACH-1625-related radioactivity in the rat and use an in vitro model of biliary clearance to estimate human ACH-1625 biliary clearance. Finally, we identify individual Cytochrome P-450 (CYP) isoforms involved in the metabolism of ACH-1625 using recombinant human CYP enzymes.

These early qualitative assessments explore the potential of ACH-1625 as a "victim" of a pharmacokinetic interaction with co-administered drugs. These initial queries will guide the design of further kinetic studies.

FIGURE 1. Mechanisms of Hepatocellular Drug Transport (left) and Rat Liver and Plasma Concentrations of ACH-1625 (right)



Adapted from ref 1

Methods

1) Hepatic extraction: ACH-1625 absorption and hepatic extraction (E_h) was determined in the rat after oral administration of 2, 10, 50, 200 and 600 mg/kg. Sprague-Dawley rats were surgically prepared by catheter insertion into portal and jugular veins. ACH-1625 plasma concentrations were measured by LC-MS/MS. In a separate study, the effect of atorvastatin (4 mg/kg, iv) on the hepatic extraction of ACH-1625 (20 mg ACH-1625/kg) was studied. Uptake of ^{14}C-ACH-1625 into isolated hepatocyte suspensions was measured after incubation with ^{14}C-ACH-1625.

2) Hepatobiliary transport: The biliary excretion of ACH-1625 was studied in primary rat and human hepatocytes where cells are grown in a 3-dimensional substrate (sandwich cultures). Use of this model has distinguished compounds with low, moderate and high biliary clearance in rats and humans [2,3]. Importantly, these cells express uptake and efflux transporters. In the presence of calcium (Ca), hepatocytes rapidly polarize forming cannicular networks within the intercellular tight junctions. In Ca^- media, the formation of this network is disrupted. Triplicate cultures were incubated in Ca^+ or Ca^- for 10 min, followed by 10 min incubation with ^{14}C-ACH-1625 (1μM) in Ca^+ media. After 10 min, all cells were harvested and radioactivity assessed. Cellular accumulation in Ca^+ media is representative of drug in hepatocytes and biliary space. The tight junctions are disrupted in Ca^- media and cellular accumulation represents only the drug in hepatocytes; drug in "bile" is released to the media. The difference in cellular accumulation between the two incubation conditions was used to calculate intrinsic clearance and scaled to in vivo biliary clearance using the well-stirred model.

3) CYP reaction phenotype profiling: Human recombinant CYP enzymes were used to determine the contribution of the individual CYP isoforms to the overall metabolic clearance of ACH-1625 in humans. The intrinsic clearance (CL_{int}) for each isoform was calculated from the rate of substrate loss and normalized for its abundance in human liver microsomes to determine the relative contribution of each CYP to the overall P450 metabolism [4]

4) Transporter Interaction Studies: The interaction of ACH-1625 with specific transporters was investigated in a screening study. Interaction with ATP-dependant family of transporters (MDR1, BCRP, MRP2) was studied using insect cell membranes inserted with each transporter. The generation of inorganic phosphate as a measure of ATP hydrolysis was measured to define the interaction with ACH-1625 in the presence of activators or inhibitors. Other assays (MRP2) measured the inhibition of probe substrate transport by ACH-1625.

Results

1 ACH-1625 SHOWS HIGH HEPATIC EXTRACTION IN THE RAT

TABLE 1. ACH-1625 Hepatic Extraction in Rats Following Oral Administration of ACH-1625

	Dose (mg/kg) [HED, (mg)]				
	2 [50]	10 [112]	50 [560]	200	600
Jugular PK					
AUC (ng-hr/mL)	24	125	9291	312182	500748
DNAUC	12	12.5	186	1560	834
E_h (%)	0.90	0.898	0.673	0.155	0.108
Portal PK					
AUC (ng-hr/mL)	239	1235	28360	369532	561510
DNAUC	120	124	567	1847	935

DNAUC = dose normalized AUC

$E_h = (DNAUC_{portal} - DNAUC_{jugular}) / DNAUC_{portal}$

FIGURE 2. ACH-1625 Jugular and Portal Vein Plasma Concentration-time Profiles after an Oral Dose (20 mg ACH-1625/kg)

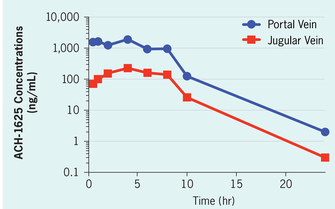
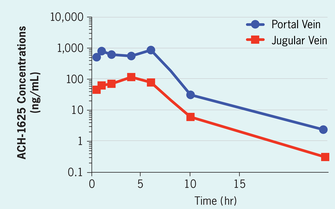


FIGURE 3. ACH-1625 Jugular and Portal Vein Plasma Concentration-time Profiles after an Oral Dose (20 mg ACH-1625/kg) in the Presence of Atorvastatin



- ACH-1625 plasma concentrations increase greater than proportional to dose from 2 to 600 mg/kg.
- In the dose proportional range (2–10 mg/kg) gut absorption is linear and systemic exposure is limited by hepatic extraction (>90%).
- At higher doses, hepatic extraction is clearly saturated (E_h decreases to 0.155 and 0.108) and exposure becomes absorption dependant.
- As shown in Figures 2 and 3, the presence of atorvastatin (3500 ng-hr/mL) does not influence the hepatic extraction of ACH-1625.

2 ACH-1625 SHOWS HIGH HUMAN HEPATOCYTE UPTAKE AND INTERACTS WITH HUMAN EFFLUX TRANSPORTERS

FIGURE 4. Active Uptake of ACH-1625 into Rat and Human Hepatocytes

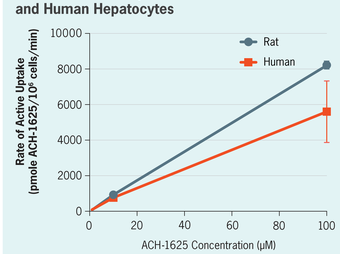


TABLE 2: Summary of ACH-1625 Interaction with Efflux Proteins Involved with Hepatobiliary Transport

Transporter	Interaction	
	Substrate	Inhibitor
BCRP	yes	no
MRP2	TBD	yes
MDR1	yes	yes
Rat MRP2	yes	yes

- ACH-1625 shows linear active uptake from 1 to 100 μM.
- This indicates a high capacity for uptake that is unlikely to be affected by co-administered drugs.
- Screening assays with biliary efflux transporters shows that ACH-1625 can be transported into bile via at least two human efflux transporters.

3 ACH-1625 SHOWS EXTENSIVE BILIARY ELIMINATION

FIGURE 5. Excretion Pathways of ^{14}C-ACH-1625 in the Rat After an iv Dose (5 mg/kg)

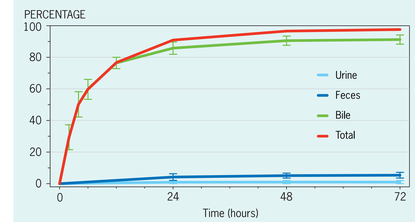


FIGURE 6. Radiochromatogram of ^{14}C-ACH-1625 Metabolites in Rat Bile Following an Oral Dose of 20 mg/kg

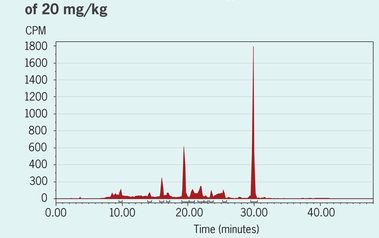


FIGURE 7. Distinction of Accumulation (Uptake) and "Biliary" Elimination in Sandwich-Cultured Rat Hepatocytes

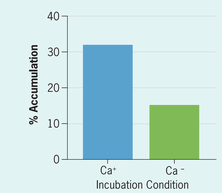


TABLE 3. Prediction of in Vivo Biliary Clearance from Hepatocyte Sandwich Cultures

Species	Predicted $CL_{biliary}$ (mL/min/kg	% HBF
Rat	44	80
Human	10	50

- After an intravenous dose of 5 mg/kg to the rat, 91% of the administered dose was recovered in bile showing extensive biliary elimination of parent drug and metabolites. Parent drug was the major single component of rat bile.
- Calculated biliary clearance of total radioactivity in vivo was 55 mL/min/kg and correlates with the value predicted from rat hepatocyte sandwich cultures (44 mL/min/kg).
- Clearance predictions from the human sandwich-cultured hepatocyte assays show a moderate biliary clearance of about 10 mL/min/kg or ~ 50% of human hepatic blood flow.

4 ACH-1625 IS METABOLIZED BY SEVERAL HUMAN CYP ENZYMES

TABLE 4. Metabolism of ACH-1625 by Recombinant Human CYP Isoforms

	CYP Isoform					
Metabolic Activity	**2C8**	**2C9**	**2C19**	**2D6**	**3A4**	**3A5**
CL_{int} (μL/min/pmol CYP)	0.111	0.151	0.183	0.174	0.9	0.454
% Contribution	5.1	9.1	2.3	1.2	69	13

- Table 4 shows that ACH-1625 is metabolized predominantly by CYP3A4/5 and to a lesser degree by CYP 2C8, 2C9, CYP2C19 and 2D6. Overall, microsomal clearance was less than one-half of liver blood flow (data not shown).

Conclusions

- Hepatic extraction ratio in the rat was ≥0.9 after oral administration of human equivalent doses from 22 – 560 mg and was saturated at higher doses. Under the conditions of this study, the extensive hepatic extraction was not affected by the presence of an OATP substrate, atorvastatin.
- In the rat, excretion of drug-related radioactivity was almost exclusively via bile with parent drug as the major single component. Similar reliance on biliary elimination is expected for excretion of the drug in humans.
- While ACH-1625 is metabolized predominantly by CYP3A, other isoforms provide alternative metabolic clearance pathways.
- These qualitative estimates of hepatic clearance show redundancy and imply that the risk of ACH-1625 being a "victim" of drug-drug interaction is not high.
- These studies support the continued development of ACH-1625 in combination with standard of care, or in combination with other direct acting anti-viral agents.

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