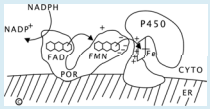
Assessment of the Hepatic Cytochrome P450 Reductase Null Mouse Model: Effect on Clearance and Exposure of Docetaxel, Midazolam, Nelfinavir and Theophylline

Jason W. Boggs[1], Edna F. Choo[1], Anna Dornier[1], Jennifer Harris[1], Ann Qin[1], Michael Reich[2], Cornelis Hop[1]
[1]Departments of Drug Metabolism and Pharmacokinetics, [2]In-Vivo Studies, Genentech Inc., South San Francisco, CA, 94080, USA

INTRODUCTION

- P450 Oxidoreductase (Por) is the essential electron donor for all P450 enzymes and is responsible for the activation of P450 metabolism (see Figure 1).
- The Taconic Hepatic Cytochrome P450 (CYP) Reductase Null (HRN) Mouse Model possesses a targeted mutation that results in liver-specific deletion of the Por gene disrupting P450 metabolism in the liver.
- This model could be useful in assessing new chemical entities (NCE) for proof of concept studies, e.g., in vivo efficacy, where high hepatic CYP clearance (CL) and low exposure may preclude in vivo evaluation.
- The objective of our studies was to characterize the HRN mouse model by administering probe drugs and by observing changes in pharmacokinetics of HRN mice compared to wild-type (WT) animals.

Figure 1 – Relationship of POR to a microsomal cytochrome P450 enzyme



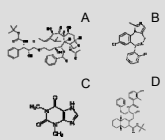
Huang N et al. PNAS 2008;105:1733-1738

MATERIALS & METHODS

In Vivo PK studies:

- Female HRN Mice (Taconic, NY) & C57BL6 (WT; Taconic, Oxnard) were used for all studies.
- Commercially available Docetaxel (DTX), Midazolam (MDZ), Nelfinavir (NFV) and Theophylline (TPL) were administered by intravenous (IV) bolus injection into the tail vein; Midazolam was also administered via Oral Gavage (PO).
  - Probe drugs were selected to reflect CL by different human CYP's and to reflect low (TPL, 1A2), medium (MDZ, 3A4) and high (DTX, 3A4/A5) CL drugs. NFV was selected to reflect mixed CL pathways (transporters and CYP3A4, 2C19) (see Figure 2 for structures of drugs).
  - Doses (5 mL/kg) used for the study were 2 mg/kg: MDZ & TLP and 5 mg/kg: DTX & NFV.
  - Aminobenzotriazole (ABT, 50 mg/kg; PO) was administered PO (10 mL/kg) 2 hours prior to administration of the drug of interest to inhibit CYP activity (extra-hepatic CYP in HRN mice or both hepatic and extra-hepatic CYP in WT mice).
- Two blood samples (70 µL retro-orbital, 200 µL terminal) were collected per mouse at specified time points (n=3 samples/time point) using K_2EDTA as anticoagulant.
- Blood sample collection times were at either; 0.083, 0.167, 0.5, 1, 3, 6, 9, 24 hr or 0.083, 0.25, 0.5, 1, 4, 8 hr.
- Plasma samples were analyzed using LC/MS/MS.
- Mean plasma concentrations were calculated for each timepoint and non-compartmental analysis was conducted on the mean concentration data using WinNonlin v5.2.

Figure 2 – Chemical structures of A) Docetaxel B) Midazolam C) Theophylline and D) Nelfinavir



RESULTS AND DISCUSSION

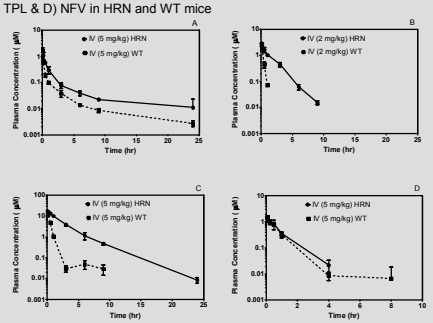
1. Comparison of PK in HRN and WT mouse following IV Administration.

- Compared to WT animals, a 2.1-, 4.5- and 5.2-fold reduction in CL was observed for DTX, MDZ & TPL, respectively. Volume of distribution was unchanged (Table 1; Figure 3).
- A corresponding 1.3-, 6.8- and 2.7-fold increase in half-life was observed for DTX, MDZ & TPL, respectively (Table 1; Figure 3).
- CL of NFV was very similar in HRN and WT mice following IV dosing which suggests that non CYP or extra-hepatic CYP pathways are involved in NFV's CL.

Table 1: Summary of Preclinical PK Parameters following IV bolus administration of DTX, MDZ, TPL or NFV

Drug	Dose (mg/kg)	Strain	AUC0-inf (hr*µM)	CL (mL/min/kg)	$t_{1/2}$ (hr)	Vss (L/kg)
DTX	5	HRN	1.59	58.4	11.0	25.8
		WT	0.801	124	8.06	28.0
MDZ	2	HRN	3.91	8.52	1.23	0.878
		WT	0.865	38.5	0.182	0.575
TPL	2	HRN	37.3	4.96	2.55	0.779
		WT	7.23	25.6	0.995	0.958
NFV	5	HRN	1.19	123	0.675	6.82
		WT	1.16	126	0.957	6.77

Figure 3 – IV bolus mean plasma concentration-time profiles of A) DTX B) MDZ C) TPL & D) NFV in HRN and WT mice



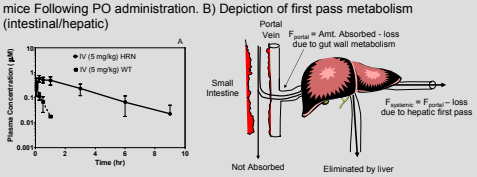
2. Comparison of MDZ Oral Exposure between HRN and WT Mouse

- The oral AUC of MDZ was increased by ~20-fold in HRN mice compared to WT mice (Table 2, Figure 4).
- The greater difference observed between HRN and WT mouse following PO dosing compared to IV dosing (20-fold increase in AUC) suggests that hepatic first pass plays a large role in the oral CL of MDZ.

Table 2: Summary of Preclinical PK Parameters Following PO Administration of MDZ

Drug	Dose (mg/kg)	Strain	AUC0-inf (hr*µM)	Cmax (µM)	Tmax (hr)
MDZ	2	HRN	1.75	0.618	0.250
		WT	0.0843	0.269	0.0833

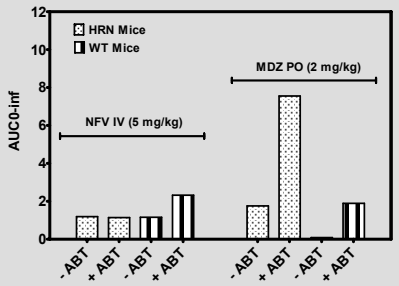
Figure 4 – A) Mean plasma concentration-time profile of MDZ in HRN and WT mice Following PO administration. B) Depiction of first pass metabolism (intestinal/hepatic)



3. Co-administration of with ABT (NFV & MDZ)

- To determine the contribution of extra-hepatic CYP metabolism to CL of NFV and MDZ in HRN mice, ABT was co-administered with NFV & MDZ.
- Relatively small difference in NFV PK with ABT co-administration further suggests that non-CYP (hepatic or extra-hepatic) pathways are involved in NFV CL (later confirmed in vitro ~20% turnover in mouse liver microsomes; Nelfinavir is also a substrate of P-gp) (Figure 5).
- Approximately 4-fold increase in MDZ exposure in ABT treated HRN mice compared to non treated mice suggests that extra-hepatic CYP plays a role in MDZ CL.
- ~20-fold increase in WT mice when administered with ABT indicates that extra-hepatic CYP metabolism plays a role in MDZ CL less significant role for MDZ metabolism in HRN mice vs. WT mice.

Figure 5 – NFV & MDZ AUC0-inf in HRN & WT mice following IV or PO administration with or without ABT



SUMMARY AND CONCLUSIONS

- Liver-specific deletion of the Por gene disrupts CYP450 metabolism in the liver thereby reducing the hepatic CL of drugs that are primarily eliminated by CYP450.
- Drugs with a range of CL and metabolized by different CYP450 isoenzymes are affected by the lack of hepatic CYP450.
- In addition, HRN mice ±ABT is useful in discerning the contribution of extra-hepatic metabolism (e.g. intestinal CYP450 metabolism) to the total CL of a drug.
- Further characterization of possible compensatory CL pathways may be necessary to determine differences in HRN vs. WT mice.
- Overall, the HRN mouse model could potentially be a valuable tool in evaluating tool compounds in drug discovery where high hepatic CL result in low bioavailability and exposure.
- The HRN mouse model would be less useful for increasing exposure of compounds where extra-hepatic metabolism plays a major role in CL.

References

•Henderson CJ, Otto DME, Carrie D, Magnuson MA, McLaren AW, Rosewell I, Wolf CR. (2003) Inactivation of the hepatic cytochrome P450 system by conditional deletion of hepatic cytochrome P450 reductase. J Biol Chem 278(15):13480-13486.

•Huang N, Agrawal V, Giacomini KM, Miller WL 2008 Genetics of P450 oxidoreductase. Sequence variation in 842 individuals of four ethnicities and activities of 15 missense mutations. Proc Natl Acad Sci USA 105:1733–1738

Acknowledgements

We thank Murray Cutler, the IVS and Small Molecule Pharmaceutics group in their assistance in the generation of the data.

Genentech
IN BUSINESS FOR LIFE