Drug Metabolism Disposition

In vivo **imaging of human *MDR1* transcription in the brain and spine of *MDR1-luciferase* reporter mice**

Kazuto Yasuda, Cynthia Cline, Yvonne S. Lin, Rachel Scheib, Samit Ganguly, Ranjit Thirumaran, Amarjit Chaudhry, Richard B. Kim and Erin G. Schuetz

Supplemental Methods

Rationale for drug and dose selection. Dexamethasone 5 mg/kg was chosen because this dose induced mouse BBB Pgp (Bauer et al., 2004), and because this dose of dexamethasone in mice would give an exposure level (Bauer et al., 2004) therapeutically relevant to human exposure (Szymanska et al., 2012). 200 mg/kg DEX was chosen because that dosing regimen maximally induced mouse PXR target genes (Wrighton et al., 1995). Although dexamethasone dosing regimens in humans can vary significantly depending on disease and patient condition during therapy, the 200 mg/kg dose would be expected to achieve a plasma concentration many fold higher than therapeutic human plasma concentrations. Rifampin 15 mg/kg was chosen because it was reported that an ip dose of 10 mg/kg resulted in exposure in mice similar to that in humans receiving a standard dose of these drugs, and that the plasma unbound fraction of RIF was similar to that in humans, and that an ip dose greater than 10 mg/kg was needed to induce hepatic PXR targets in wild-type mice (Hasegawa et al, 2011; Scheer et al., 2008). Rifampin 50 mg/kg was selected because this oral dose induced mouse BBB Pgp, and achieved peak plasma levels of free drug similar to those in patients taking single, daily 6.4 mg/kg doses (448-mg dose for 70-kg

patient) (Bauer et al., 2006). Since we administered 50 mg/kg rifampin ip, the mouse rifampin plasma concentration is many fold higher than therapeutic human plasma concentration. A dose of 40 mg/kg Phenobarbital was chosen because this dose readily activates CAR to induce target genes (Scheer et al., 2008). Phenobarbital 80 mg/kg induced mouse BBB Pgp (Wang et al., 2010). The average therapeutic plasma concentration for phenobarbital in human is reported to be 10- 40 µg/ml (Loscher, 2007; Markowitz et al., 2010). In a reported intravenous pharmacokinetic study of 60 mg/kg phenobarbital in male mice, the C_0 for phenobarbital was measured to be close to 75 µg /ml (Liu et al., 2007). Assuming an oral bioavailability of 80% (Nelson et al., 1982) and a dose –linear exposure at lower doses (Loscher, 2007; Geter et al., 2014), we can assume the C_0 for a 40 mg/kg intravenous dose of phenobarbital would be approximately 50 µg/ml. This plasma concentration is on the higher end of the phenobarbital therapeutic range. Considering pharmacokinetic variability due to gender and species of animals, we can assume that at 40 mg/kg i.p., our study was conducted within the therapeutic concentration range. TCPOBOP is not given clinically, treatment dose and schedule were selected based on maximal activation of CAR and induction of BBB Pgp (Wang et al., 2010)(Scheer et al., 2008).

Supplemental Table 1. Doses Chosen for This Study Compared to Published Studies

This Study				Published study		
	Dose	Duration	Figure	Dose	Duration	Ref
TCPOBOP	3 mg/kg	daily x 4	2	0.33 mg/kg 1 - 30 mg/kg	daily x 2 24 hrs	(Wang et al., 2010) (Scheer et al., 2008)
DEX	200 mg/kg 200 mg/kg	daily x 2 5hrs	3 - 5 4	250 mg/kg	daily x 4	(Wrighton et al., 1995)
	5 mg/kg	daily x 3	4, 5	1–50 mg/kg	daily x 3	(Bauer et al., 2004)
PB	40 mg/kg 40 mg/kg	5hrs daily x 2	3, 4 4 - 7	80 mg/kg (rat) 40 mg/kg	daily x 4 daily x 4	(Wang et al., 2010) (Scheer et al., 2008)
Rifampin	15 mg/kg 50 mg/kg	daily x 2 daily x 4	4 5	1-60 mg/kg 50 mg/kg*	daily x 4 1-3 days	(Scheer et al., 2008) (Hasegawa et al., 2011) (Bauer et al., 2006)
Elacridar	100 mg/kg*	4 hrs	3 - 6	100 mg/kg*	0-24 hrs	(Sane et al., 2010)

*, oral; all other drugs given ip

Supplemental Data References

Bauer B, Hartz AM, Fricker G, and Miller DS (2004) Pregnane X receptor up-regulation of P-glycoprotein expression and transport function at the blood-brain barrier. *Mol Pharmacol* **66:**413-419.

Bauer B, Yang X, Hartz AM, Olson ER, Zhao R, Kalvass JC, Pollack GM, and Miller DS (2006) In vivo activation of human pregnane X receptor tightens the blood-brain barrier to methadone through P-glycoprotein up-regulation. *Mol Pharmacol* **70:**1212-1219.

Geter DR, Bhat VS, Gollapudi BB, Sura R, and Hester SD (2014) Dose-response modeling of early molecular and cellular key events in the CAR-mediated hepatocarcinogenesis pathway. *Toxicological Sci* **138:**425-445.

Liu H, Zhang D, Xu X, Liu X, Wang G, Xie L, Pang X, and Liu L (2007) Attenuated function and expression of P-glycoprotein at blood-brain barrier and increased brain distribution of phenobarbital in streptozotocin-induced diabetic mice. *Euro Jnl Pharmacol* **561:**226-232.

Loscher W (2007) The pharmacokinetics of antiepileptic drugs in rats: consequences for maintaining effective drug levels during prolonged drug administration in rat models of epilepsy. *Epilepsia* **48:**1245-1258.

Markowitz GJ, Kadam SD, Boothe DM, Irving ND, and Comi AM (2010) The pharmacokinetics of commonly used antiepileptic drugs in immature CD1 mice. *Neuroreport* **21:**452-456.

Nelson E, Powell JR, Conrad K, Likes K, Byers J, Baker S, and Perrier D (1982) Phenobarbital pharmacokinetics and bioavailability in adults. *J Clin Pharamcol* **22:**141-148.

Sane, R, Agarwal, S and Elmquist, WF (2012) Brain distribution and bioavailability of elacridar after different routes of administration in the mouse. *Drug Metab Dispos* **40**: 1612-1619.

Scheer N, Ross J, Rode A, Zevnik B, Niehaves S, Faust N, Wolf CR (2008)
A novel panel of mouse models to evaluate the role of human pregnane X receptor and constitutive androstane receptor in drug response. *J Clin Invest.* **118:**3228-39.

Hasegawa M, Kapelyukh Y, Tahara H, Seibler J, Rode A, Krueger S, Lee DN, Wolf CR, Scheer N (2011) Quantitative prediction of human pregnane X receptor and cytochrome P450 3A4 mediated drug-drug interaction in a novel multiple humanized mouse line. *Mol Pharmacol*. **80**:518-28.

Szymanska B, Wilczynska-Kalak U, Kang MH, Liem NL, Carol H, Boehm I, Groepper D, Reynolds CP, Stewart CF, and Lock RB (2012) Pharmacokinetic modeling of an induction regimen for in vivo combined testing of novel drugs against pediatric acute lymphoblastic leukemia xenografts. *PloS ONE* **7:**e33894.

Wang X, Sykes DB, Miller DS (2010) Constitutive androstane receptor-mediated up-regulation of ATP-driven xenobiotic efflux transporters at the blood-brain barrier. *Mol Pharmacol* **78**:376-83.

Wrighton SA, Schuetz EG, Watkins PB, Maurel P, Barwick J, Bailey BS, Hartle HT, Young B, Guzelian PS (1985) Demonstration of multiple species of inducible hepatic cytochromes P-450 and their mRNAs related to the glucocorticoid-inducible cytochrome P-450 of the rat. *Mol Pharmaco*l **28**:312-321.