Application of Micropatterned Co-cultured Hepatocytes to Evaluate the Inductive Potential and Degradation Rate of Major Xenobiotic Metabolizing Enzymes

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Supplemental Table 1: Summary of Rifampin drug related input parameters used for Simcyp modeling. * Data Source: Simcyp compound library v13.

Parameter*	Rifampin
Mol Weight (g/mol)	823
Log P	3.28
pKa	7.9, 1.7
$f_{u, plasma}$	0.15
Blood/plasma ratio (B/P)	0.9
f_a	1
Absorption rate constant: k_a (1/h)	0.51
Volume of distribution: V_{ss} (L/kg)	0.33
Renal clearance: CL_R (L/h)	1.2
Systemic plasma clearance: CL_{iv} (L/h)	7

Supplemental Table 2: Comparative statistics (n=3) of the gene expression in HepatoPac model vs the monoculture model. ΔCt values from both models were compared using t test in GraphPad Prism. P value of less than 0.05 was considered statistically significant.

Taqman Probe	Metabolic enzymes	p value NON	p value BPB	p value Hu1624
AOX1-Hs00154079_m1	aldehyde oxidase	0.04	0.79	
CYP1A1-Hs00153120_m1	CYP1A1	0.16	0.01	0.52
CYP1A2-Hs00167927_m1	CYP1A2	0.03	0.38	0.01
CYP2A6-Hs00868409_s1	CYP2A6	0.01		0.97
CYP2B6-Hs04183483_g1	CYP2B6	0.05	0.00	0.53
CYP2C19-Hs00426380_m1	CYP2C19	0.13	0.01	0.01
CYP2C8-Hs00946140_g1	CYP2C8	0.01	0.00	0.06
CYP2C9-Hs02383631_s1	CYP2C9	0.07	0.02	0.63
CYP2D6-Hs03043788_g1	CYP2D6	0.13	0.00	0.00
CYP2E1-Hs00559368_m1	CYP2E1	0.00	0.00	0.00
CYP3A4-Hs00604506_m1	CYP3A4	0.00	0.00	0.25
CYP3A43-Hs01119078_mH	CYP3A43	0.27		0.07
CYP3A5-Hs01070905_m1	CYP3A5	0.17	0.00	0.81
CYP3A7-Hs00426361_m1	CYP3A7	0.80	0.00	0.01
CYP4F12-Hs02515808_s1	CYP4F12	0.03	0.02	0.06
FMO1-Hs00266654_m1	FMO1	0.30	0.03	0.32
FMO2-Hs00155158_m1	FMO2	0.16		
FMO3-Hs00199368_m1	FMO3	0.14	0.23	0.87
FMO4-Hs00157614_m1	FMO4	0.00	0.01	0.01
FMO5-Hs00356233_m1	FMO5	0.39	0.13	0.04
FOXO1-Hs01054576_m1	FOXO1	0.12	0.76	0.10
GSTA2-Hs00747232_mH	GST A2	0.12	0.41	0.07
GSTA3-Hs02555455_sH	GST A3	0.03	0.84	0.13
SULT1A1-Hs00738644_m1	SULT1A1		0.31	0.07
SULT1A2-Hs02340929_g1	SULT1A2	0.07	0.04	0.02
SULT1B1-Hs00234899_m1	SULT1B1	0.00	0.00	0.00
SULT1E1-Hs00960941_m1	SULT2E1	0.01	0.12	0.02
SULT2A1-Hs00234219_m1	SULT2A1	0.01	0.04	0.07
UGT1A1-Hs02511055_s1	UGT1A1	0.01	0.00	0.01
UGT1A6-Hs01592477_m1	UGT1A6	0.00	0.29	0.81
UGT1A8-Hs01592482_m1	UGT1A8	0.01	0.02	0.01
UGT2B15-Hs00870076_s1	UGT2B15	0.37	0.00	0.16
UGT2B4-Hs02383831_s1	UGT2B4	0.04	0.01	0.10
UGT2B7-Hs00426592_m1	UGT2B7	0.07	0.01	0.06
CYP2J2-Hs00951113_m1	CYP2J2	0.02	0.95	0.65
AADAC	AADAC	0.000015	0.001926	0.000203
CES	CES	0.000216	0.009267	0.000041

Taqman Probe	Transporters	p value NON	p value BPB	p value Hu1624
ABCB1-Hs00184500_m1	MDR1	0.21	0.01	0.00
ABCB11-Hs00184824_m1	BSEP	0.02	0.03	0.00
ABCB4-Hs00240956_m1	MDR3	0.02	0.00	0.11
ABCC1-Hs01561502_m1	MRP1	0.00	0.04	0.49
ABCC2-Hs00166123_m1	MRP2	0.03	0.99	0.01
ABCC3-Hs00978473_m1	MRP3	0.52	0.01	0.05
ABCC4-Hs00988717_m1	MRP4	0.76	0.10	0.09
ABCC6-Hs00184566_m1	MRP6	1.00	0.68	0.00
ABCG2-Hs01053790_m1	BCRP	0.99	0.01	0.01
SLC10A1-Hs00161820_m1	NTCP	0.19	0.05	0.10
SLC22A1-Hs00427552_m1	OCT1	0.03	0.01	0.65
SLC22A7-Hs00198527_m1	OAT2	0.73	0.00	0.09
SLCO1B1-Hs00272374_m1	OATP1B1	0.04	0.16	0.57
SLCO1B3-Hs00251986_m1	OATP1B3	0.00	0.00	0.01
SLCO2B1-Hs01030343_m1	OATP2B1	0.00	0.00	0.02
SLC47A1-Hs00217320_m1	MATE1	0.84	0.03	0.03
SLC29A1-Hs01085704_g1	ENT1	0.01	0.00	0.00
SLC29A2-Hs00155426_m1	ENT2	0.75	0.00	0.04

Taqman Probe	Nuclear receptors	p value NON	p value BPB	p value Hu1624
AHR-Hs00169233_m1	AhR	0.13	0.67	0.67
HNF1A-Hs00167041_m1	HNF 1 alpha	0.08	0.14	0.69
HNF4A-Hs00230853_m1	HNF 4 alpha	0.96	0.02	0.12
NCOA1-Hs00186661_m1	nuclear receptor coactivator 1	0.02	0.18	0.86
NCOA2-Hs00896106_m1	nuclear receptor coactivator 2	0.23	0.01	0.92
NCOA3-Hs01105251_m1	nuclear receptor coactivator 3	0.11	0.00	0.78
NCOR1-Hs01094540_m1	nuclear receptor corepressor 1	0.00	0.00	0.00
NCOR2-Hs00196955_m1	nuclear receptor corepressor 2	0.07	0.00	0.30
NR0B2-Hs00222677_m1	SHP1	0.02	0.08	0.06
NR1H2-Hs01027208_m1	LXR	0.76	0.03	0.35
NR1H4-Hs01026590_m1	FXR	0.08	0.50	0.26
NR1I2-Hs01114267_m1	PXR	0.04	0.13	0.02
NR1I3-Hs00901571_m1	CAR1	0.03	0.01	0.57
NR3C1-Hs00353740_m1	glucocorticoid receptor	0.00	0.37	0.84
PPARGC1A-Hs01016719_m1	PPARg	0.53	0.02	0.01
RXRA-Hs01067640_m1	RXRa	0.14	0.12	0.34
VDR-Hs00172113_m1	VDR	0.92	0.45	0.35

FIGURE LEGENDS:

Supplemental Figure 1: cDNA from vehicle treated cells from both monocultures and HepatoPac cultures of hepatocytes were used to determine the basal expression of transcription factors, as well as co-activator and co-repressor genes. Gene expression in HepatoPac cultures were compared with monocultured hepatocytes and the data were expressed as relative change over monocultured cells in 3 lots of hepatocytes. Data are shown as mean (SD) of 3 determinations.

Supplemental Data Figure 1