

A Comparison of Adult and Neonatal Human Hepatocytes in Drug Metabolizing Enzyme Activities

Albert P. Li, David Ho, and Kirsten Amaral

In Vitro ADMET Laboratories Inc., Columbia, MD, and Malden, MA

*Corresponding author: Albert P. Li, Ph. D. (lialbert@invitroadmet.com)

Scientific Rationale

- While it is generally accepted that neonates and adults differ in drug metabolizing enzyme activities, there is little information on the differences in specific drug metabolizing enzyme pathways
- In our laboratory, hepatocytes were isolated and cryopreserved from three neonates with encephalopathy who had full-term *in utero* development, borne alive, and survived for minutes to hours after birth due to the lack of brain functions
- We report here drug metabolizing enzyme activities of hepatocytes isolated and cryopreserved from three neonatal donors

Materials & Methods

- Neonatal livers were obtained from IIAM (Edison, NJ)
- Hepatocytes were isolated via collagenase digestion, cryopreserved, and stored in liquid nitrogen
- The cryopreserved neonatal hepatocytes were thawed, recovered in UCRM (IVAL, Columbia, MD) and resuspended in Hepatocyte Incubation Medium (IVAL) for incubation with pathway selected substrates for the determination of DME activities

Demographics of Neonatal Liver Donors

Lot No.	Gestational Period	Gender	Race
HH1066	39 weeks	Female	Not Reported
HH1129	41 weeks	Female	Caucasian
HH1130	39 weeks	Male	Asian

Drug Metabolism Enzyme Pathways Evaluated

Metabolic Pathway	Substrate	Substrate Conc. (μ M)	Marker Metabolite
CYP1A2	Phenacetin	100	Acetaminophen
CYP2A6	Coumarin	50	7-HC, 7-HC-Sulfate, 7-HC-Glucuronide
CYP2B6	Bupropion	500	Hydroxybupropion
CYP2C8	Paclitaxel (Taxol)	20	6 α -hydroxypaclitaxel
CYP2C9	Diclofenac	25	4-OH Diclofenac
CYP2C19	S-Mephenytoin	250	4-OH S-Mephenytoin
CYP2D6	Dextromethorphan	15	Dextrophan
CYP2E1	Chlorzoxazone	250	6-OH Chlorzoxazone
CYP3A4-1	Midazolam	20	1-Hydroxymidazolam
CYP3A4-2	Testosterone	200	6 β -hydroxytestosterone
ECOD	7-Ethoxycoumarin	100	7-HC, 7-HC-Sulfate, 7-HC-Glucuronide
UGT	7-Hydroxycoumarin	100	7-Hydroxycoumarin Glucuronide
SULT	7-Hydroxycoumarin	100	7-Hydroxycoumarin Sulfate
GST	Acetaminophen	10 mM	Acetaminophen Glutathione
FMO	Benzylamine HCl	250	Benzylamine-N-Oxide
MAO	Kynuramine HBr	160	4-hydroxyquinoline
AO	Carbazepin	10	4-Hydroxycarbazepin
NAT1	4-Aminobenzoic Acid	200	N-Acetyl-p-aminobenzoic acid
NAT2	Sulfamethazine	100	N-Acetyl-sulfamethazine

Results

DME Pathway	Activity (pmol/min/million cells)										NEONATAL/ADULT RATIO (%)
	HH1066		HH1129		HH1130		Mean of Three Neonatal Lots		Adult Hepatocytes PHH8001		
	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD	
CYP1A2	0.0	0.0	0.0	0.0	0.000	0.000	0.00	0.00	58.52	10.46	0.00
CYP2A6	0.184	0.099	0.283	0.107	0.132	0.053	0.20	0.08	102.21	7.55	0.20
CYP2B6	0.272	0.077	0.328	0.107	0.085	0.058	0.23	0.13	29.42	2.43	0.78
CYP2C8	0.368	0.244	0.523	0.207	0.013	0.005	0.30	0.26	141.13	23.31	0.21
CYP2C9	6.951	0.844	2.278	0.105	3.458	0.155	4.23	2.43	97.63	6.34	4.33
CYP2C19	0.000	0.112	0.000	0.082	0.000	0.000	0.00	0.00	19.74	2.08	0.00
CYP2D6	2.694	0.311	0.204	0.051	0.285	0.010	1.06	1.42	17.26	1.70	6.15
CYP2E1	0.844	0.737	0.422	0.301	17.226	1.749	6.16	9.58	47.22	1.02	13.05
CYP3A4-1	2.273	0.054	3.535	0.116	1.632	0.229	2.48	0.97	15.68	0.39	15.81
CYP3A4-2	17.956	2.501	27.311	2.983	16.919	0.614	20.73	5.72	204.04	34.29	10.16
ECOD	1.871	0.058	2.293	0.162	4.840	0.342	3.00	1.61	72.66	9.43	4.13
UGT	8.802	1.772	2.773	0.328	10.828	0.741	7.47	4.19	537.00	19.53	1.39
SULT	3.917	0.914	4.142	0.164	2.270	0.424	3.44	1.02	19.97	1.42	17.24
GST	6.169	0.744	6.473	0.760	2.854	1.893	5.17	2.01	25.36	2.15	20.37
FMO	8.527	1.267	4.216	0.684	13.029	0.441	8.59	4.41	761.33	44.85	1.13
MAO	42.867	5.553	46.622	3.222	47.665	7.377	45.72	2.52	1892.67	94.52	2.42
AO	0.287	0.094	0.233	0.036	0.085	0.005	0.20	0.10	0.93	0.06	21.64
NAT1	7.156	0.473	9.889	1.059	2.627	0.112	6.56	3.67	9.20	7.68	71.24
NAT2	0.338	0.390	0.744	0.080	0.000	0.039	0.36	0.37	41.51	3.36	0.87

Summary and Conclusions

- Hepatocytes of three neonatal livers were evaluated for drug metabolizing enzymes and compared to that of pooled donor adult human hepatocytes (5 males and 5 females)
- Neonatal hepatocytes DME activities with activity ranged from 0 to 71% of that of the adult hepatocytes
 - <5% for 9 pathways (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, UGT, FMO, MAO, NAT2)
 - >5% for CYP2D6 (6%), CYP2E1 (13%), CYP3A4 (16% and 10%), SULT (17%), GST (20%), AO (22%) and NAT1 (71%)

The results suggest that dosing regimen to neonatals should take into account of their substantially different DME activities from adults, especially for drugs metabolized by the 9 pathways that are <5% of the adults