CYP3A Induction in Cynomolgus Monkey and Human Hepatocytes

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Introduction:

Non-human animals such as mouse, rat, rabbits and dogs are significantly different from humans in response to P450 inducers because of their differences in the expression of drug metabolizing enzymes and species-dependent differences in substrate specificities compared to man. This species-difference has led to the lack of a generally-accepted preclinical animal model for the evaluation of this specific aspect of pharmacokinetic drug-drug interactions. We report here our evaluation of cynomolgus monkeys as a potentially relevant nonhuman animal species for the evaluation of human drug-drug interactions, with emphasis on CYP3A induction.

Materials and Methods:

• **Chemicals**: All chemicals used in this study were purchased from Sigma-Aldrich Co.(St. Louis, MO). All the cells and cell culture materials and medium were purchased from ALIVE and A.P.Sciences Inc.

• **Primary Hepatocyte Isolation and Culture**: Cryopreserved human and pooled male monkey (cynomolgus) hepatocytes were used for the study. Pooled monkey hepatocytes were prepared by isolating hepatocytes from 50 male monkey livers using two-step collagenase digestion procedure. The hepatocytes were pooled immediately after isolation and then cryopreserved using established procedures. Human hepatocytes were isolated from individual donors and then cryopreserved. Cells used for the study had >90% viability and plating efficiency. Throughout the study the cells were maintained in 5% CO2/95% highly humidified air.

• **CYP3A Induction**:

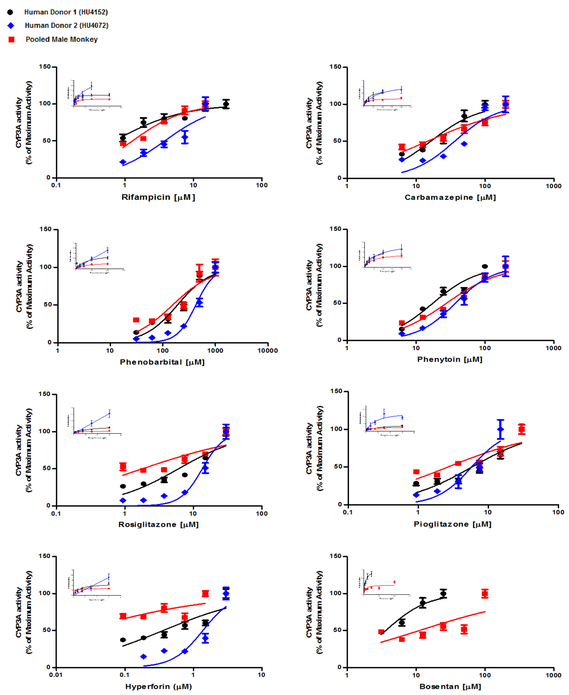
Activity: Cryopreserved pooled male monkey hepatocytes and human hepatocytes from individual donor were plated at a density of 12500 cells/well by adding 10 µL to a collagen coated 384-well plate and were allowed to attach for 4h. Following attachment the cells were overlayed with 0.25 mg/ml of Matrigel™ and were incubated overnight. Next day plating medium was replaced with Hepatocyte Induction Medium containing prototypical inducers [Rifampicin (30-0.7µM), Pioglitazone (30-0.7µM), Rosiglitazone (30-0.7µM), Hyperforin (3-0.07µM), Carbamazepine (200-6.1µM), Phenobarbital ((1000-31.2µM), Omeprazole (50-1.5 µM), Phenytoin (200-0.7µM) and Bosentan ((100-3.1 µM)] and was incubated for an additional 72hrs. Following treatment the cells were washed twice with Hepatocyte Metabolism Medium and then incubated with 25µL of 3µM Luciferin-IPA (Promega, WI). After 1hr of incubation 20µL was removed and transferred to a white plate and luminescence was measured using Victor Wallac Multi-label plate reader by adding equal amount of Luciferin detection reagent (Promega). Cellular viability was assessed by measuring ATP content using ATPlite kit (Perkin Elmer).

Gene Expression: Cryopreserved pooled male monkey hepatocytes and male hepatocytes from individual donor were plated in a collagen coated 24-well plate at a cell density of 350,000 cells/well. Following 4h of attachment, plating medium was changed to a fresh plating medium containing 0.25 mg/ml of Matrigel™. After overnight incubation, cells were washed with Hepatocyte Induction Medium and then incubated in with one concentration of prototypical inducers [Rifampicin (30µM), Pioglitazone (30µM), Rosiglitazone (30µM), Hyperforin (3µM), Carbamazepine (200µM), Phenytoin (200µM) and Bosentan ((100µM)]. 1000x stock solutions of test articles were prepared in DMSO and the final concentration of DMSO in all incubations was 0.1%. Medium containing 0.1% DMSO was used as vehicle control. The cells were incubated with test articles for a total period of 72h with treatment medium changed to fresh medium every 24h. Following treatment cells were harvested and mRNA was isolated using mini RNAeasy kit (Qiagen) and manufacturer's protocol. Total RNA of 1µg from each sample was used to synthesize cDNA using iScript cDNA synthesis kit (Biorad). Primers were designed for real-time PCR and the sequences will be provided upon request. Real time-reactions were carried out using SYBR Green PCR master mix (Applied Biosystems) using the ABI 7500 Real-time PCR system (Applied Biosystems). Expression levels of mRNA were normalized to GAPDH. Relative expression was calculated by comparing to vehicle control and the values were plotted as fold induction.

• **Data Analysis**: All activity results were expressed as the mean of quadruplicate determinations, whereas those of gene expression were expressed as mean of triplicate determination. The parameters Emax (maximum observed induction at an optimum concentration) and EC50 (effective concentration that shows half-maximal induction) were determined from dose response curve using following equation y=Emax [I]/(EC50 + [I]), where [I] is inhibitor concentration, and Prism Software, version 5.00 from GraphPad Software (La Jolla, CA)

Results:

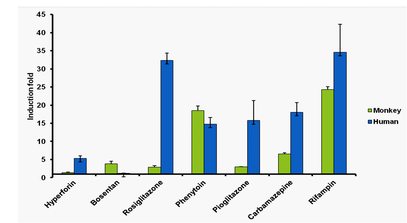
CYP3A Activity Profile of Human and Pooled Monkey Hepatocytes in Response to Inducers



	Human Donor 1 (HU4152)		Human Donor 2 (HU4072)		Pooled Monkey	
	EC_{50} µM (SE)	E_{max} fold induction (SE)	EC_{50} µM (SE)	E_{max} fold induction (SE)	EC_{50} µM (SE)	E_{max} fold induction (SE)
Rifampicin	0.74(0.16)	10.79(0.4)	8.56(4.8)	28.7(8.1)	1.4(0.19)	6.97(0.2)
Carbamazepine	27.7(6.1)	5.36(0.5)	74.5(20.6)	7.16(0.9)	12.9(2.9)	2.3(0.13)
Phenobarbital	391(86.8)	15.17(1.4)	-	-	281(89.1)	5.9(0.7)
Phenytoin	27.1 (05.5)	6.65(0.5)	73.5(22.8)	10.1(1.3)	42.3(6.6)	5.51(0.3)
Rosiglitazone	10.4(2.9)	5.9(0.7)	-	-	1.2(0.4)	1.9(0.1)
Pioglitazone	9.9(2.9)	4.7(0.6)	7.9(5.1)	13.8(3.5)	2.1(0.6)	2.5(0.2)
Hyperforin	0.48(0.14)	2.3(0.2)	-	-	0.08(0.03)	1.4(0.07)
Bosentan	5.3(1.2)	6.04 (0.5)	-	-	5.3(2.3)	2.3(0.2)

Table 1. Comparison of activity profile of CYP3A inducers in human and pooled monkey hepatocytes. The results are reported as mean with standard error (SE) in the parenthesis.

CYP3A Expression Profile in Human and Pooled Monkey Hepatocytes in Response to Inducers



Drugs	Human (Lot #HU4072)	Pool Monkey
	Fold Induction Mean ± SD	Fold Induction Mean ± SD
Rifampicin	34.6 ± 7.7	24.3 ± 0.8
Carbamazepine	18.1 ± 2.7	6.6 ± 0.3
Phenytoin	14.8 ± 1.9	18.5 ± 1.2
Rosiglitazone	32.4 ± 1.9	2.9 ± 0.4
Pioglitazone	15.7 ± 5.5	2.9 ± 0.1
Hyperforin	5.3 ± 0.7	1.4 ± 0.2
Bosentan	1.2 ± 0.1	3.8 ± 0.7

Table 2. Effects of CYP3A inducers on CYP3A mRNA expression reported as fold induction compared to vehicle control in human and pooled monkey hepatocytes

Summary of Results:

• A high throughput 384-well plate CYP3A induction assay was developed with cryopreserved human and cynomolgus monkey hepatocytes.

• Dose dependent induction in CYP3A activity was observed in human and pooled monkey hepatocytes with most of the inducers evaluated.

• Apparent differences were observed between monkey and human in the magnitude of induction, mainly due to the substantially higher basal CYP3A activity in the monkey hepatocytes.

• Gene expression results in general reflect findings based on CYP3A activity.

Conclusions:

Cynomolgus monkeys represent an appropriate preclinical animal species for the evaluation of human CYP3A induction.

Suggestion for Application of Monkey Hepatocytes in CYP3A induction:
A prudent approach would be to first compare human and monkey hepatocytes in response to the inducer to be evaluated, and, if results are determined to be similar between the two species, evaluate the inducer in monkeys in vivo before embarking upon human clinical studies.