

Evaluating the Role of Pharmacogenetics in Attention-Deficit/Hyperactivity Disorder Using Human Hepatocytes and Enzyme Inhibition in Human Liver Microsomes

Rebecca Law, MS; Daniel Hain, BS; Rachel Daut, PhD; Holly Johnson, PhD; Jerry Lanchbury, PhD

Authors were employed by Myriad Genetics, Inc. at the time of this study

INTRODUCTION

- Most medications approved to treat attention-deficit/hyperactivity disorder (ADHD) were approved over 30 years ago.
- As a result, few studies have characterized the metabolism of these medications to current standards.
- The present study aimed to characterize the metabolism of racemic amphetamine, dextroamphetamine, racemic methylphenidate, dexamethylphenidate, atomoxetine, clonidine, and guanfacine using modern *in vitro* methods.

METHODS

- First, the rates of parent loss and metabolite formation were measured over 48 hours in cryopreserved primary human hepatocytes (cPHHs) and over 2 hours in human liver microsomes (HLMs).
- Second, the fraction metabolized by drug metabolizing enzymes was measured in HLMs using chemical inhibition.
- Experimental conditions were tested in triplicate, and positive and negative controls were included in duplicate.

- The proportion of atomoxetine, racemic methylphenidate, dexamethylphenidate, and guanfacine converted to various metabolites largely matched *in vivo* reports.
- There were some major pathways that were not observed: oxidative deamination and beta-hydroxylation of racemic amphetamine and dextroamphetamine, and no major loss of clonidine was observed.
- Well-characterized and robust gene-drug interactions (GDIs), such as atomoxetine with *CYP2D6* and guanfacine with *CYP3A4/5*, were replicated in the present study.
- In addition, previously reported GDIs were not replicated and potentially novel GDIs were observed.

- Contrary to recent reports that propose *CYP2C19* forms N-desmethylatomoxetine, *CYP2C19* inhibition did not significantly reduce the formation of N-desmethylatomoxetine in this study and its formation was minimal in cPHHS and HLMs.
- Additionally, after administration of methylphenidate, *CYP2B6* and *CYP2D6* inhibition largely reduced the area ratio of oxo-methylphenidate and p-hydroxy-methylphenidate, suggesting a novel role for these enzymes.

RESULTS

Table 1. Percent substrate remaining, half-life, and estimated *in vitro* intrinsic clearance in cPHHs and percent substrate remaining in HLMs

Substrate	Measured Compound	48hr in cPHHs			2hr in HLMs
		Percent Remaining	T _{1/2} estimated from exponential decay (min)	Estimated <i>in vitro</i> CL _{int} (μL/min/million cells)	Percent Remaining
D/L-Amphetamine	D-Amphetamine	97.9%	>2880	<0.185	98.4%
	L-Amphetamine	99.1%	>2880	<0.185	92.8%
Dextroamphetamine	Dextroamphetamine	No Loss	>2880	<0.185	95.4%
Clonidine	Clonidine	No Loss	>2880	<0.185	93.6%
D/L-Methylphenidate	D-Methylphenidate	0%	217	>2.22	89.5%
	L-Methylphenidate	0%	72.9	>2.22	87.3%
Dexamethylphenidate	Dexamethylphenidate	0%	289	1.85	76.5%
Atomoxetine	Atomoxetine	0%	217	>2.22	13.1%
Guanfacine	Guanfacine	69.6%	>2880	<0.185	80.1%

Table 2. Amount of metabolite formation in cPHHs and HLMs after administration of 1000pmol of substrate

Substrate	Measured Compound	48hr in cPHHs		2hr in HLMs
		Average peak detected (pmol)	Time of peak detected (minutes)	Average detection at 2hr in no solvent control (pmol)
D/L-Amphetamine	4-OH-amphetamine	8.74	1440	5.75
	Norephedrine	2.55	1440	Not Formed
Dextroamphetamine	4-OH-amphetamine	6.04	2880	3.22
	Norephedrine	2.32	1440	Not Formed
Clonidine	4-OH-clonidine	17.3	2880	30.2
	4-OH-atomoxetine	56.0	240	513
Atomoxetine	4-OH-atomoxetine-O-glucuronide	984	1440	241
	N-desmethyl-atomoxetine	14.9	240	13.5 ^A
Guanfacine	3-OH-guanfacine	6.63	480	149

Metabolites were only included 1. if reference standards were available and if standard curves were created to measure amount of a substance and 2. if the formation of the metabolite was enzyme-mediated and not due to non-enzymatic degradation
^Astandard curve failed in the no solvent control, average represents the average across 4 solvent controls

CONCLUSIONS

- The present study represents the first modern characterization of the intrinsic clearance of many ADHD medications.
- Additionally, the current findings highlight the need for further research to fully understand the impact of GDIs and pharmacogenetics, particularly for medications that were approved prior to the development of more advanced *in vitro* techniques.

Table 3. Percent inhibition of parent loss and metabolite formation after a 2-hour incubation with HLMs

Substrate	Measured Compound	Retention Time (Minutes)	3A4/5	1A2	2B6	2C9	2C19	2D6
D/L-Amphetamine	4-OH-amphetamine	NA	N.I.	N.I.	55.6	3.3	N.I.	36.8
Dextroamphetamine	4-OH-amphetamine	NA	5.7	N.I.	50.7	N.I.	6.7	≥63.1
Clonidine	4-OH-clonidine	NA	N.I.	11.2	31.4	N.I.	3.1	74.6
D/L-Methylphenidate	p-hydroxy-methylphenidate	3.12	18.9	47.9	16.7	39.8	8.6	N.I.
		3.43	36.0	N.I.	34.5	42.2	21.2	30.9
		3.65	12.2	12.3	74.1	3.3	37.2	66.6
		4.05	65.3	N.I.	24.9	N.I.	N.I.	57.4
	Oxo-methylphenidate	4.25	100	N.I.	N.F.	3.5	19.5	23.6
Dexamethylphenidate	p-hydroxy-methylphenidate	4.80	20.9	N.I.	58.9	14.5	38.5	57.7
		2.92	40.9	N.I.	27.2	N.F.	N.I.	14.5
		3.27	16.7	6.2	59.4	38.3	19.7	40.7
		3.48	N.I.	5.8	55.3	24.6	15.2	42.2
	Oxo-methylphenidate	3.88	46.4	19.1	2.4	2.7	N.I.	17.6
Atomoxetine	Atomoxetine	4.16	54.0	20.4	16.9	18.5	42.3	40.3
		4.68	16.1	27.4	63.6	20.1	32.1	56.3
	4-OH-Atomoxetine	NA	N.I.	5.7	45.0	12.4	N.I.	100
	4-OH-Atomoxetine-O-Glucuronide	NA	N.I.	N.I.	30.6	N.I.	N.I.	93.0
	N-desmethyl-atomoxetine	NA	24.8	14.4	N.I.	7.6	9.1	N.I.
	N-desmethyl-hydroxy-atomoxetine	NA	5.3	4.8	45.4	N.I.	8.1	100
	2-hydroxy-atomoxetine	3.23	N.I.	5.8	36.5	N.I.	35.2	43.5
Guanfacine	Guanfacine	4.4	38.7	5.5	N.I.	48.1	27.3	N.I.
		5.62	12.6	N.I.	N.I.	70.9	6.5	N.I.
	3-OH-guanfacine	NA	100	12.9	N.I.	3.6	10.4	7.8

Enzyme (Chemical Inhibitor): *CYP3A4/5* (ketoconazole), *CYP1A2* (furafylline), *CYP2B6* (phencyclidine), *CYP2C9* (tienilic acid), *CYP2C19* (esomeprazole), and *CYP2D6* (paroxetine). N.I. = no inhibition, N.F. = not formed, NA = not applicable

Findings of 30% and higher are green.

- Parent loss only reported where at least 10% of the substrate is lost in the no solvent control condition (table 1) and where metabolite formation requires active enzymes, as shown with the negative controls of no cofactor addition and no protein addition. For that reason, amphetamine, dextroamphetamine, clonidine, methylphenidate, dexamethylphenidate, and ritalinic acid results are not given.