IN VITRO ASSESSMENT OF THE PHARMACOKINETIC DRUG-DRUG INTERACTION POTENTIAL OF RASAGILINE AND ITS MAJOR METABOLITE AMINOINDAN

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Introduction

Rasagiline mesylate (PAI) is the active pharmaceutical ingredient of the anti-Parkinson's drug Azilect, marketed by Teva Neuroscience, Inc. Rasagiline (R-N-2-Propynyl-1-indanamine) is a second generation, selective and irreversible inhibitor of monoamine oxidase (MAO)-B. Prior to FDA approval in 2006, the metabolism of rasagiline to its major metabolite aminoindan (AI) by CYP1A2 was characterized in vitro by traditional reaction phenotyping approaches and in a follow-up clinical study where the AUC of rasagiline (2 mg/day) increased by 83% when co-dosed with the strong CYP1A2 inhibitor ciprofloxacin (500 mg b.i.d.) (Azilect label). Additionally, the potential for rasagiline to cause direct and/or metabolism-dependent inhibition of cytochrome P450 (CYP450) enzymes was evaluated; rasagiline did not inhibit any of the CYP450 enzymes tested (CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4/5 and CYP4A11).

In the current study, we further evaluated rasagiline and its major human metabolite, aminoindan (Figure 1), for the potential to be the perpetrator or victim of pharmacokinetic based drug-drug interactions. The experimental procedures were based on the recommendations and scientific principles described in the FDA DDI draft guidance for industry (2012), the EMA guideline on the investigation of drug interactions (2013) and in the 2014 Japanese MHLW DDI draft guidance. Rasagiline and aminoindan were tested for the potential to cause induction of CYP450 enzymes (CYP1A2, CYP2B6, CYP3A4/5) and direct and/or metabolism-dependent inhibition of CYP450 enzymes (rasagiline, CYP2B6, CYP2C8 and CYP3A4/5; aminoindan, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4/5) . Furthermore, the potential of rasagiline to be a substrate (P-gp and BCRP) and rasagiline and aminoindan to be inhibitors (P-gp, BCRP, OATP1B1, OATP1B3, OCT2, OAT1, OAT3, MATE1 and MATE2-K) of drug transporters was evaluated in vitro.

Materials & Methods

Chemicals and Reagents: Rasagiline mesylate, [^{14}C]-Rasagiline mesylate and aminoindan were provided by Teva Pharmaceuticals Industries LTD. All reagents and solvents were of analytical grade.

CYP Induction:
Three preparations of cryopreserved human hepatocytes were then treated once daily for 3 consecutive days with medium containing 0.1% v/v DMSO (vehicle control), one of three concentrations of PAI (0.05, 0.5 or 5 µM), one of three concentrations of AI (0.015, 0.15 or 1.5 µM) or one of three known human CYP enzyme inducers, namely, omeprazole (50 µM), phenobarbital (750 µM) and rifampin (20 µM), positive controls or the non-inducer, flumazenil (25 µM, negative control).
After treatment, enzymatic activity was measured in situ prior to collection of cells for mRNA isolation. First, the cells were incubated with marker substrates for CYP1A2, CYP2B6 and CYP3A4, namely, phenacetin (100 µM) bupropion (500µM) and midazolam (30 µM) for 45 min. Metabolite formation was monitored by LC-MS/MS analysis . Following the in situ incubation, the same hepatocytes from the same treatment groups were harvested with Buffer RLT to isolate RNA, which was analyzed by qRT PCR to assess the effect of PAI and AI on mRNA levels.

CYP Inhibition:
To evaluate PAI as a direct, time-dependent and metabolism-dependent inhibitor of CYP activity, pooled (n = 200) human liver microsomes were incubated with marker substrates in the presence or absence of PAI. To distinguish between time dependent and metabolism dependent inhibition, PAI was preincubated with human liver microsomes for 30 minutes without and with an NADPH-generating system, respectively, prior to the incubation with the marker substrates. Experimental details are described in Parkinson *et al*., 2011 and summarized in Table 1.

Table 1: CYP inhibition incubation conditions

Enzyme	Positive control inhibitor		Enzyme reaction	[Sub] (µM)	[Protein] (µg/mL)	Pre inc (min)	Inc (min)
	Direct (µM)	Metabolism Dependent (µM)					
CYP1A2	α-Naphthoflavone (0.5)	Furafylline (2)	Phenacetin *O*-dealkylation	90	100	0 min 30 min – NADPH 30 min + NADPH	5
CYP2B6	Orphenadrine (750)	Phencyclidine (30)	Efavirenz 8-hydroxylation	5	100		
CYP2C8	Montelukast (0.05)	Gemfibrozil glucuronide (5)	Amodiaquine *N*-dealkylation	2	12.5		
CYP2C9	Sulfaphenazole (2)	Tienilic acid (0.25)	Diclofenac 4′-hydroxylation	12	100		
CYP2C19	Modafinil (400)	Esomeprazole (10)	*S*–Mephenytoin 4′-hydroxylation	60	100		
CYP2D6	Quinidine (5)	Paroxetine (1)	Dextromethorphan *O*-demethylation	10	100		
CYP3A4/5	Ketoconazole (0.075)	Troleandomycin (7.5)	Testosterone 6β-hydroxylation	60	100		
			Midazolam 1′-hydroxylation	3	50		

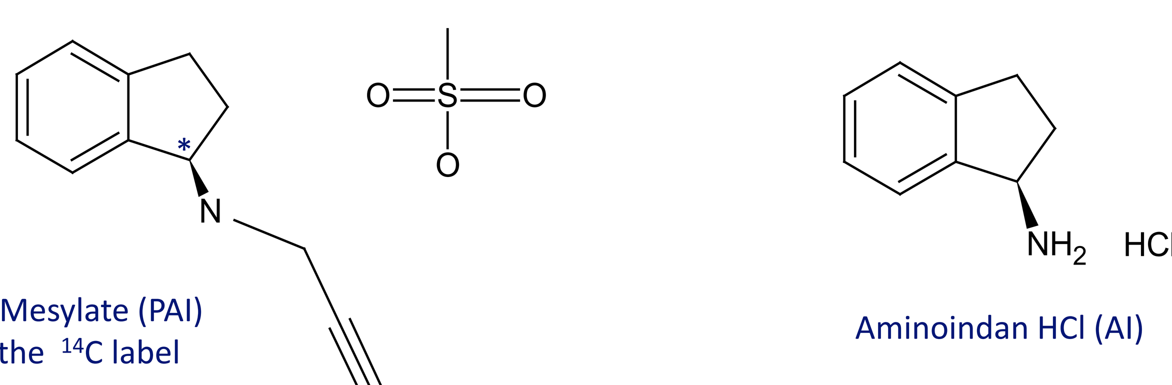
Transporter inhibition:
The ability of PAI (30 - 5000 nM) and AI (1 - 300 nM) to inhibit human efflux transporters, namely, P-gp and BCRP was evaluated by measuring the bidirectional permeability of a probe substrates (digoxin or prazosin) across a monolayer of Caco-2 and MDCKII -BCRP cells grown on 24-well trans well plates in the presence of PAI and AI. Donor and receiver samples were analyzed by LC-MS/MS. The ability of PAI (3 - 3000 nM or 1 - 400 nM) and AI (0.3 - 300 nM) to inhibit human uptake transporters, namely, OATP1B1, OATP1B3, OCT2, OAT1, MATE1 and MATE2-K was evaluated by measuring the accumulation of probe substrates in transporter-expressing and control HEK293cells. At the end of the incubation, cells were rinsed three times then lysed NaOH and the radioactive probe substrate was quantified by liquid scintillation counting (MicroBeta[2]). Experimental details are summarized in Table 2.

Transporter Substrate:
To determine if [^{14}C]-PAI is a substrate of human efflux transporters (P-gp and BCRP), the bidirectional permeability of [^{14}C]-PAI (0.3, 3 and 30 µM) across MDCKII-MDR1 and MDCKII-BCRP cells was measured in the presence and absence of the P-gp inhibitor valspodar and BCRP inhibitor Ko143, respectively. [^{14}C]-PAI in the donor and receiver samples was quantified by LSC (MicroBeta2).

Table 2: Transporter incubation conditions

Transporter	Test system	Probe substrate	(µM)	Pre inc (min)	Inc time (min)	PC inhibitor 1	(µM)	PC inhibitor 2	(µM)	Analysis
P-gp	Caco-2	Digoxin	10	NA	120	Valspodar	1	Verapamil	60	LC-MS/MS
BCRP	MDCKII-BCRP	Prazosin	1	NA	120	Ko143	1	Rironavir	50	
OATP1B1	HEK293	^{3}H-Estradiol glucuronide	0.05	15	2	Rifampin	10	Cyclosporine	1	LSC
OATP1B3	HEK293			15	2					LSC
OCT2	HEK293	^{14}C-Metformin	10	15	2	Quinidine	300	Cimetidine	1000	LSC
OAT1	HEK293	p-Aminohippurate	1	15	1	Probenecid	100	Novobiocin	300	LSC
OAT3	HEK293	^{3}H-Estrone sulfate	0.05	15	2	Probenecid	100	Ibuprofen	100	LSC
MATE1	HEK293	^{14}C-Metformin	10	15	5	Cimetidine	10	Cimetidine	100	LSC
MATE2-K	HEK293	^{14}C-Metformin	10	15	5	Pyrimethamine	0.3	Pyrimethamine	0.3	LSC

Figure 1: Chemical structures

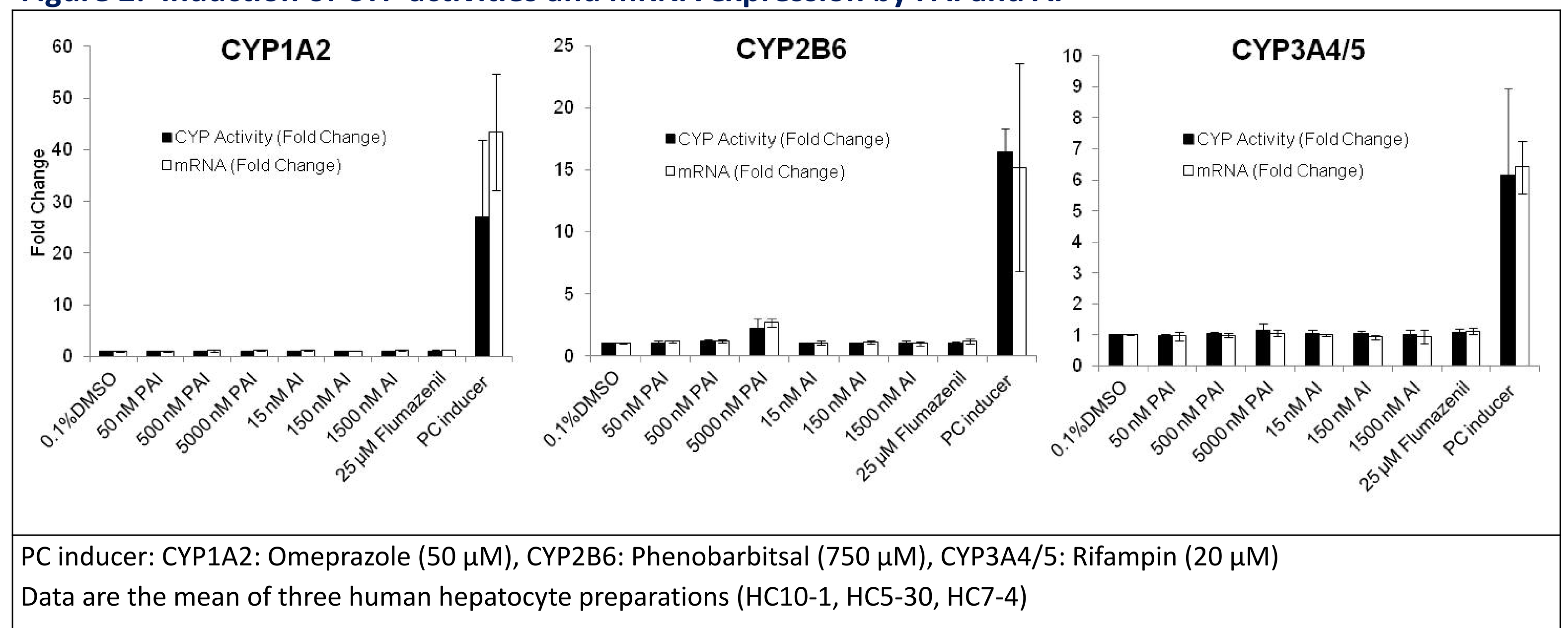


Rasagiline Mesylate (PAI)
* denotes the ^{14}C label

Aminoindan HCl (AI)

Results

Figure 2: Induction of CYP activities and mRNA expression by PAI and AI



PC inducer: CYP1A2: Omeprazole (50 µM), CYP2B6: Phenobarbitsal (750 µM), CYP3A4/5: Rifampin (20 µM)
Data are the mean of three human hepatocyte preparations (HC10-1, HC5-30, HC7-4)

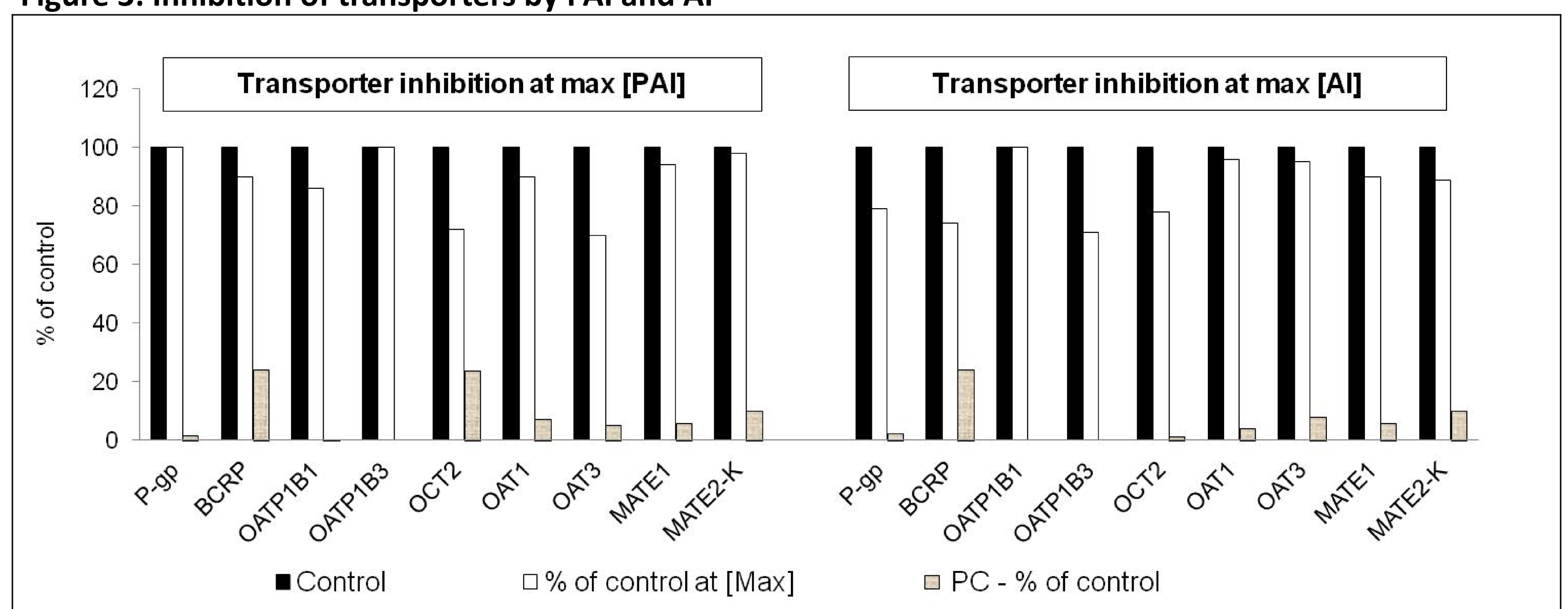
Figure 2 shows the induction of cytochrome P450 activity and mRNA expression in cultured human hepatocytes (n = 3) treated with PAI, AI or positive control inducers. The highest concentration of PAI (5000 nM) induced CYP2B6 activity 2.2-fold and mRNA expression 2.7-fold (shaded) as compared to the positive control inducer, phenobarbital which caused an increases in CYP2B6 mRNA expression by 16.4- and 15.2-fold, respectively. Induction of CYP1A2, CYP2B6 and CYP3A4 activity and mRNA was less than 2-fold at all other conditions tested.

Table 3: Direct, time dependent and metabolism dependent inhibition of CYP enzymes by PAI and AI

Enzyme	Substrate	[range] (nM)	Direct inhibition 0 min PI	TDI 30 min PI - NADPH	MDI 30 min PI +NADPH	Potential for TDI or MDI
			% inhib at [max]	% inhib at [max]	% inhib at [max]	
		PAI				
CYP2B6	Efavirenz	0, 3, 10, 30, 100, 300, 900, 3700	1.2	NA	5.8	Little or no
CYP2C8	Amodiaquine		NA	NA	3.9	Little or no
CYP3A4/5	Midazolam		3.8	NA	1.6	Little or no
		AI				
CYP1A2	Phenacetin	0, 1, 3, 10, 30, 100, 300, 400	NA	2.0	0	Little or no
CYP2B6	Efavirenz		NA	1.5	4.4	Little or no
CYP2C8	Amodiaquine		0	NA	15	Little or no
CYP2C9	Diclofenac		NA	NA	NA	Little or no
CYP2C19	*S*-Mephenytoin		4.9	0.2	16	Little or no
CYP2D6	Dextromethorphan		NA	NA	NA	Little or no
CYP3A4/5	Testosterone		NA	6.3	9.1	Little or no
CYP3A4/5	Midazolam		0.8	3.2	6.5	Little or no

Table 3 shows the results of the CYP inhibition experiments. Inhibition of CYP enzymes did not exceed 16% at the concentrations of PAI and AI tested. There was not evidence of time-dependent or metabolism-dependent inhibition.

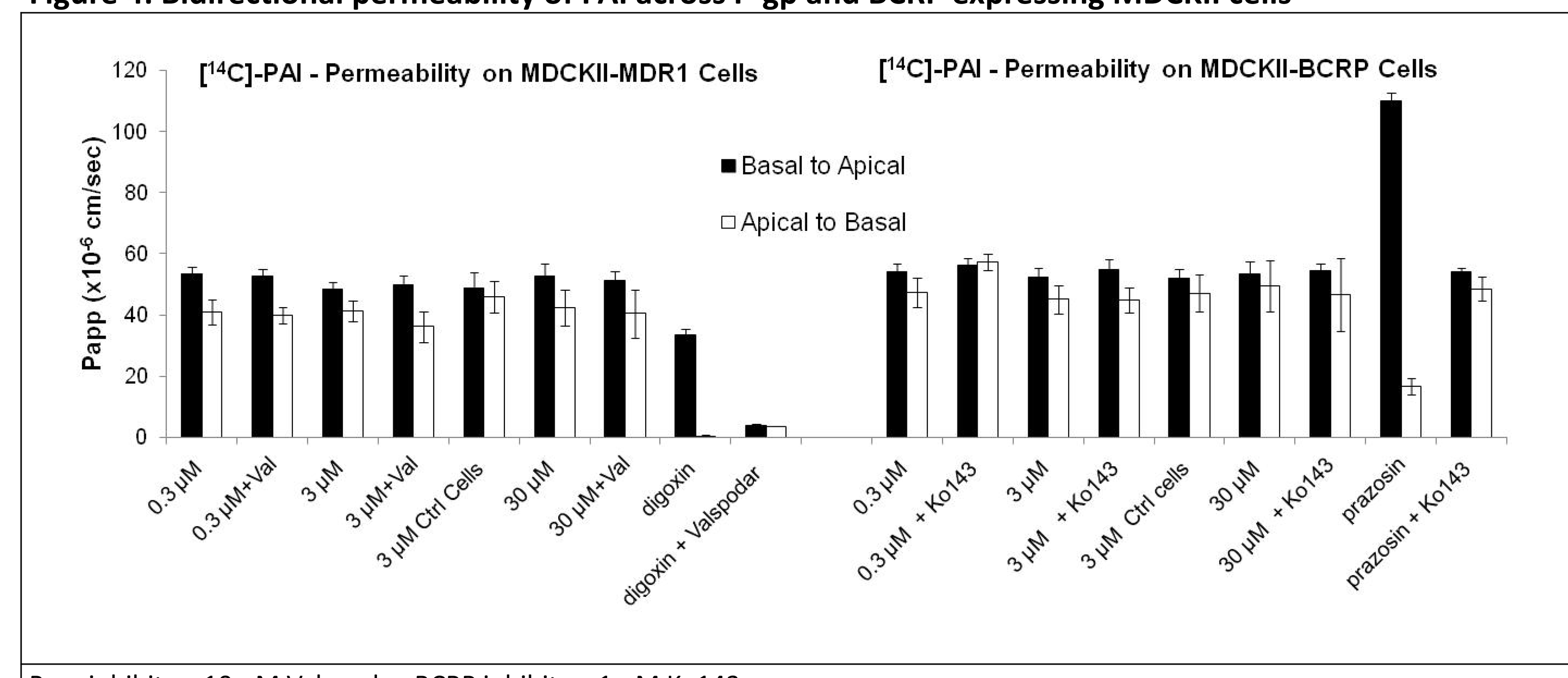
Figure 3: Inhibition of transporters by PAI and AI



[PAI] range: P-gp and BCRP: 0, 30, 100, 300, 1000, 3000 and 5000 nM; OATP1B1 and OATP1B3: 0, 3, 10, 30, 100, 300, 1000 and 3000 nM; OCT2, OAT1, OAT3, MATE1 and MATE2K: 0, 1, 3, 10, 30, 100, 300 and 400 nM
[AI] range: P-gp and BCRP: 0, 1, 3, 10, 30, 100 and 300 nM;
OATP1B1, OATP1B3, OCT2, OAT1, OAT3, MATE1 and MATE2K: 0, 0.3, 1, 3, 10, 30, 100 and 300 nM

Figure 3 shows the results of the transporter inhibition assays. Inhibition of transport did not exceed 30% in the presence of the highest concentrations of PAI or AI tested.

Figure 4: Bidirectional permeability of PAI across P-gp and BCRP expressing MDCKII cells



P-gp inhibitor: 10 µM Valspodar, BCRP inhibitor: 1 µM Ko143

Figure 4 shows the results of the P-gp and BCRP transporter substrate assays. The permeability of PAI was similar in the A-B and B-A directions resulting in efflux ratios less than two at all concentrations tested. The permeability and efflux ratios were not affected by the P-gp or BCRP inhibitors valspodar or Ko143.

Conclusions

- At the concentrations of PAI and AI evaluated in this study, these compounds did not show in vitro evidence of any of the following:
  - Induction of CYP1A2, CYP2B6 or CYP3A4
  - Direct, time- or metabolism- dependent inhibitors of the CYP enzymes
  - Inhibition of drug transporters
  - Substrate of P-gp or BCRP
- Based on the results of the in vitro drug-drug interaction studies rasagiline (PAI) and its metabolite aminoindan (AI) are not expected to be perpetrators of clinical pharmacokinetic based drug-drug interactions.

References

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