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Contribution of UGT enzymes to human drug metabolism stereoselectivity: a case study of medetomidine, RO5263397, propranolol and testosterone

Supplemental Information

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Turnover of RO5263397 and RO5263396 by recombinantly expressed UGT2B10 and UGT1A4

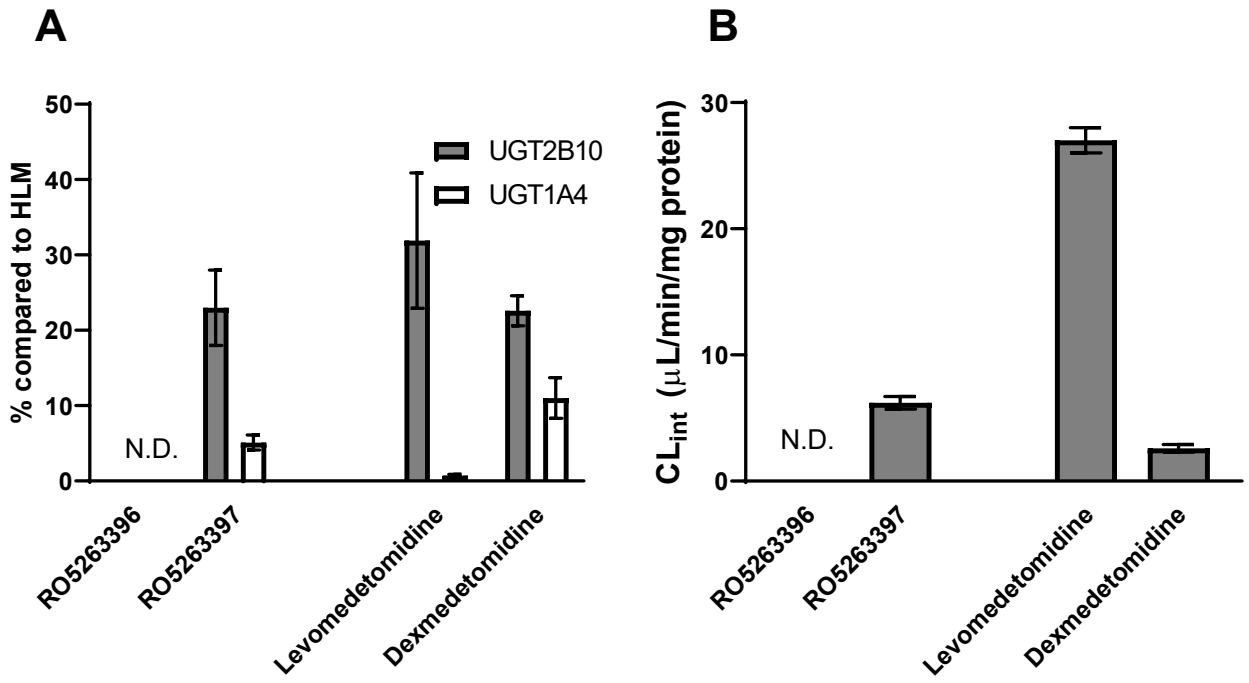
Experimental Method:

UGT2B10 and UGT1A4 were prepared in the same Tris buffer as HLM, but at a protein concentration of 0.55 mg/mL (final concentration: 0.50 mg/mL) and without the pre-treatment with alamethicin. Incubation procedure was as for UGT incubations described above; RO5263397, RO5263396 and medetomidine enantiomers were incubated at a concentration of 1µM. Sampling points were: 0.5, 3.5, 6.5, 10, 15, 30, 45, and 90 minutes and 40 µL of each incubation mixture was removed and pipetted into 384-well deep well plates preloaded with 80 µL quench solution (chilled acetonitrile containing 500 ng/mL of D_6-midazolam as internal standard).

Results:

RO5263397 has previously been shown to be selectively metabolized by UGTs and in particular by UGT2B10 (Fowler et al., 2015; Milani et al., 2020); intrinsic clearance of its enantiomer RO5263396 by UGTs was not measurable and the glucuronide formation rate was not quantifiable and it demonstrated a very high UGT metabolic stability of RO5263396 (Supplementary Information Figure S1). Levomedetomidine and dexmedetomidine were metabolized by UGT2B10 with intrinsic clearances of 27 ± 1 and 2.6 ± 0.3 µL/min/mg, respectively. (UGT1A4 Clint values were below limit of quantitation.) Therefore the most active isomer UGT2B10 metabolized levomedetomidine 10-fold faster than dexmedetomidine. Conversely, the metabolism of dexmedetomidine was indicated by the metabolite peak formation rates to be 2-fold faster than that of levomedetomidine in recombinant UGT1A4 incubations.

Figure S1. Activity of UGT2B10 and UGT1A4 enzyme preparations in the metabolism of RO5263397/6 and Medetomidine



A) Activity of UGT2B10 and UGT1A4 for RO5263396/7 and medetomidine enantiopairs as percentage of clearance in the HLM.

B) CL_{int} in the recombinant UGT2B10 incubations.

N.D. not detectable since it was below the limit of quantification (1 µL/min/mg)

Supplemental Table 1: Measured Intrinsic Clearances of Medetomidine, RO5263397, Propranolol and Testosterone in Liver Microsomes without BSA Supplementation

Compound		Medetomidine			RO5263397/6			Propranolol			Testosterone		
		Levo	Dex	Ratio	RO5263397	RO5263396	Ratio	S	R	Ratio	Epi	Test	Ratio
		µL/min/mg			µL/min/mg			µL/min/mg			µL/min/mg		
Human	UDPGA*	120 ±9	14 ±2	8.6	36 ±1	BLQ	> 36	BLQ	BLQ	ND	353 ±5	105 ±4	3.4
	NADPH	6.0 ±3.2	8.5 ±1.9	0.49	BLQ	BLQ	ND	18 ± 1	105 ±7	0.17	11 ±3	14 ±2	0.79
	Sum	1	22.5	5.6	36	BLQ	36	18	105	0.17	364	119	3.1
Cynomolgus	UDPGA	BLQ	BLQ	ND	BLQ	BLQ	ND	1.9 ± 0.7	1.2 ± 0.5	1.6	>1000	161 ±6	>6.2
	NADPH	6.2 ±1	20 ±1.6	0.31	BLQ	4.3 ±1.9	<0.23	240 ±28	>1000	<0.24	793 ±30	273 ±8	2.9
Rat	UDPGA	BLQ	BLQ	ND	BLQ	BLQ	ND	BLQ	BLQ	ND	19 ±2	245 ±8	0.076
	NADPH	10 ±1	22 ±1	0.45	BLQ	12 ±5	<0.083	>1000	852 ±71	>1.2	>1000	890 ±86	>1.1
Mouse	UDPGA	BLQ	BLQ	ND	BLQ	BLQ	ND	BLQ	BLQ	ND	3.9 ± 2.3	60 ±5	0.065
	NADPH	19 ±3	15 ±3	1.3	BLQ	6.9 ±6.0	<0.14	72 ±7	208 ±15	0.35	333 ±36	402 ±17	0.812
Dog	UDPGA	4.1 ±1.2	BLQ	>4.1	BLQ	BLQ	ND	4.7 ±0.9	BLQ	>4.7	34 ±3	843 ±42	0.040
	NADPH	9.6 ±2.7	16 ±4	0.60	10 ± 4	8.7 ±3.7	1.1	186 ±13	156 ±23	1.2	116 ±6	127 ±5	0.91
Minipig	UDPGA	BLQ	BLQ	ND	BLQ	BLQ	ND	BLQ	3.3 ±0.6	<0.30	23 ±2	63 ±1	0.40
	NADPH	8.4 ±2.0	10 ±1	0.84	BLQ	6.4 ±4.0	<0.16	>1000	360 ±43	>2.8	185 ±9	125 ±6	1.5

*Relative rates of initial glucuronide formation by pooled HLM assuming equal LC-MS/MS response of glucuronides: RO5263397/RO5263396: 74; S-Propranolol/R-Propranolol: 6.6 (see also Supplemental Table 2)

notes: sum only calculated for man since the rest is descriptive; values not calculated where CL_{int} < 1 µL/min/mg; values are a starting point

BLQ: not determined since the CL_{int} was below the limit of quantification (1 µL/min/mg)

ND: not determined

Supplemental Table 2: Measured (Bound) intrinsic Clearance and Calculated Unbound Intrinsic Clearances of Medetomidine, RO5263397, Propranolol and Testosterone in Incubations with Pooled HLM in presence and absence of BSA.

	CL_{int}						**Glucuronide Peak Appearance Rate (Peak Area Glucuronide/Peak Area Internal Standard per minute)**					
	Medetomidine µL/min/mg			**Epi/Testosterone** µL/min/mg			**RO5263396/7**			**Propranolol**		
	Levo	Dex	Ratio[a]	Epi	Test	Ratio[a]	S	R	Ratio[a]	S	R	Ratio[a]
- BSA observed	120	14	8.6	353	104	3.4	0.35	0.0047	74	0.011	0.0017	6.6
+ BSA observed	96	11	8.7	597	43	14	0.56	0.0043	130	0.085	0.012	7.0
- BSA free fraction corrected	235	26	9.0	559	149	3.8	0.51	0.0067	77	0.085	0.0044	6.6
+ BSA free fraction corrected	300	34	8. 7	5012	424	12	0.82	0.0059	139	0.40	0.029	14
ratio CL_{int}+BSA/-BSA[b]	0.80	0.79		1.7	0.41		1.6	0.91		7.6	7.1	
ratio unbound CL_{int}+BSA/-BSA[c]	1.3	1.3		9.0	2.5		1.6	0.89		14	6.6	

[a] Ratio between CL_{int} or $CL_{int,u}$ for the respective enantiomers

[b] Effect of BSA for each enantiomer on the CL_{int}

[c] Effect of BSA for each enantiomer on the $CL_{int,u}$

Supplemental Table 3: Glucuronide formation ratio of S and R propranolol in presence and absence of BSA in HLM , HIM, and HKM.

		Glucuronide formation rate					
Enantiomer	Condition	HLM	Ratio	HIM	Ratio	HKM	Ratio
S-propranolol	No BSA	0.0112 ± 0.003	7.6	$7.4\ 10^{-4} \pm 1\ 10^{-5}$	5.0	0.0062± 0.0003	12
	BSA	0.085 ± 0.001		0.0037± 0.0001		0.076± 0.002	
R-propranolol	No BSA	0.00170 ± 0.00051	7.1	$2.4\ 10^{-4} \pm 1\ 10^{-5}$	6.5	0.00124± 0.00007	12
	BSA	0.0121 ± 0.0002		0.00157± 0.00003		0.0153±0.0003	

Supplemental Table 4: MS/MS parameters and retention time for all enantiomers

Parent				Metabolite			DP (Volts)	CE (Volts)
Compound	**Chromatography***	**Q1-Q3**	**RT** (min)	**Q1-Q3**	**Conf.**	**RT** (min)		
Medetomidine	A	201.0-94.9	0.65	377.0-201.0	Levo	0.70	70	25
					Dex	0.68		
RO5263396/ RO5263397	B	195.1-152.1	0.58	371.1-195.1	R	0.55	76	21
					S	0.62		
Propranolol	C	260.2-116.1	0.66	436.2-260.2	R	0.67	70	25
					S	0.77		
Epi/Testosterone	B	289.2-109	1.38	465.289.2	Epi.	1.18	80	30
			1.31		Test.	1.11		

*See Table 5

Supplemental Table 5: Chromatography programs of all enantiopairs

Medetomidine (A)		
Column	ACQUITY UPLC BEH C18 Column, 130Å, 1.7 µm, 2.1 mm X 50 mm	
Column Temp. (°C)	50	
Injection volume	2 µL	
Solvent A	Water + 0.1% HCOOH	
Solvent B	AcN + 0.1% HCOOH	
Time (min)	**% Solvent B**	**Flow (mL/min)**
0	15	0.6
1.30	40	
1.31	98	1.2
1.60	98	
1.61	15	
2.00	15	0.6

RO5263396/7 & Epi_Testosterone (B)		
Column	ACQUITY UPLC BEH C18 Column, 130Å, 1.7 µm, 2.1 mm X 50 mm	
Column Temp. (°C)	50	
Injection volume	2 µL	
Solvent A	Water + 0.1% HCOOH	
Solvent B	AcN + 0.1% HCOOH	
Time (min)	**% Solvent B**	**Flow (mL/min)**
0	5	0.6
1.29	60	
1.30	98	1.2
1.60	98	
1.61	5	
2.00	5	0.6

Propranolol (C)		
Column	ACQUITY UPLC BEH C18 Column, 130Å, 1.7 µm, 2.1 mm X 50 mm	
Column Temp. (°C)	50	
Injection volume	2 µL	
Solvent A	Water + 0.1% HCOOH	
Solvent B	AcN + 0.1% HCOOH	
Time (min)	**% Solvent B**	**Flow (mL/min)**
0	15	0.6
1.30	45	
1.31	98	1.2
1.60	98	
1.61	15	
2.00	15	0.6