pharmaceutics

Supplementary Materials: Investigating the Contribution of Drug-Metabolizing Enzymes in Drug-Drug Interactions of Dapivirine and Miconazole

Guru Raghavendra Valicherla, Phillip Graebing, Junmei Zhang, Ruohui Zheng, Jeremy Nuttall, Peter Silvera and Lisa Cencia Rohan

Supplementary information

Supplementary tables:

Table S1. MRM transitions of DPV, substrates, metabolites, and internal standards.

Compound	Precursor	Product	ESI Polarity	Collision energy(eV)
Dapivirine	330.11	158.05	+	24
d4-Dapivirine (I.S.)	334.13	158.89	+	32
Phenacetin	180.1	138.05	+	10
Acetaminophen	152.1	110.05	+	16
7-Ethoxyresorufin	242.023	214.058	+	22
Resorufin	214.03	214.03	+	31
Bupropion	240.1	184.2	+	8
Hydroxybupropion	256.1	238.05	+	11
Amodiaquine	356.2	283.1	+	21
d10-Amodiaquine (I.S.)	366.2	289.2	+	20
N-Desethylamodiaquine	328.24	283.1	+	16
(S)-Mephenytoin	218.963	134.13	+	20
Hydroxymephenytoin	235.027	133.091	+	20
Midazolam	326.05	291.05	+	25

1'-hydroxymidazolam	341.94	324.08	+	25
Orphenadrine HCl (I.S.)	270.2	181.1	+	12
Telmisartan (I.S.)	515.2	276.0	+	48
Telmisartan (I.S.)	513.27	287.12	−	36
SN-38	393.10	349.05	+	25
SN-38 glucuronide	569.243	393.198	+	27
Chenodeoxycholic acid	391.3	391.3	−	8
Chenodeoxycholic acid glucuronide	567.35	113.04	−	26
Trifluoperazine	408.06	141.13	+	28
Trifluoperazine glucuronide	584.21	408.16	+	26
4-methyl umbelliferone	176.947	77.02	+	24
4-methyl umbelliferone glucuronide	353.067	177.043	+	53
Mycophenolic acid	320.98	206.94	+	34
Mycophenolic acid glucuronide	519.27	343.211	+	31
Zidovudine	266.08	223.034	+	10
Zidovudine glucuronide	444.09	126.89	+	21
Propofol glucuronide (I.S.)	353.17	177.131	−	26

Table S2. Incubation conditions used for the reaction phenotyping assays.

Enzyme	Protein concentration	Substrate concentration
rCYP1A1	100 pmol/mL	Phenacetin (10 μM)
rCYP1A2	10 pmol/mL	Phenacetin (10 μM)
rCYP1B1	10 pmol/mL	7-ethoxyresorufin (1 μM)
rCYP2B6	10 pmol/mL	Bupropion (10 μM)
rCYP2C8	10 pmol/mL	Amodiaquine (0.5 μM)
rCYP2C19	10 pmol/mL	Mephenytoin (20 μM)
rCYP3A4	10 pmol/mL	Midazolam (1 μM)
HLM (Phase 1)	1 mg/mL	Midazolam (1 μM)
rUGT1A1	0.1 mg/mL	SN38 (0.5 μM)
rUGT1A3	0.4 mg/mL	Chenodeoxycholic acid (2 μM)
rUGT1A4	0.2 mg/mL	Trifluoperazine (0.5 μM)
rUGT1A6	0.5 mg/mL	4-methylumbelliferone (2 μM)
rUGT1A7	0.075 mg/mL	4-methylumbelliferone (10 μM)
rUGT1A8	2 mg/mL	4-methylumbelliferone (2 μM)
rUGT1A9	0.1 mg/mL	Mycophenolic acid (0.2 μM)
rUGT2B7	2 mg/mL	Zidovudine (10 μM)
HLM (Phase 2)	1 mg/mL	4-methylumbelliferone (2 μM)

Table S3. Incubation conditions used for the enzyme inhibition assays.

Enzyme	Protein (concentration)	Substrate (concentration)	Incubation time (min)
CYP1A1	Recombinant (50 pmol/mL)	Phenacetin (10 μM)	15
CYP1A2	HLM (0.4 mg/mL)	Phenacetin (10 μM)	15
CYP1B1	Recombinant (5 pmol/mL)	7-ethoxyresorufin (1 μM)	10
CYP2B6	HLM (0.2 mg/mL)	Bupropion (10 μM)	15
CYP2C8	HLM (0.02 mg/mL)	Amodiaquine (0.5 μM)	5
CYP2C19	HLM (0.9 mg/mL)	Mephenytoin (20 μM)	60
CYP3A4	HLM (0.1 mg/mL)	Midazolam (1 μM)	5
UGT1A1	HLM (0.2 mg/mL)	SN38 (0.5 μM)	15
UGT1A3	HLM (0.2 mg/mL)	Chenodeoxycholic acid (2 μM)	15
UGT1A4	HLM (0.2 mg/mL)	Trifluoperazine (0.5 μM)	30
UGT1A6	Recombinant (0.2 mg/mL)	4-methylumbelliferone (2 μM)	5
UGT1A7	Recombinant (0.075 mg/mL)	4-methylumbelliferone (10 μM)	15
UGT1A8	Recombinant (1 mg/mL)	4-methylumbelliferone (2 μM)	45
UGT1A9	HLM (0.1 mg/mL)	Mycophenolic acid (0.2 μM)	10
UGT2B7	HLM (0.4 mg/mL)	Zidovudine (10 μM)	60

Table S4. Donor information of cryopreserved primary human hepatocytes used for the induction assays.

Donor information	HU2036 (Donor 1)	HU8373 (Donor 2)	HU2068 (Donor 3)
Sex	Male	Female	Male
Race	Caucasian	Caucasian	Caucasian
Age	71	26	66
BMI	27.76	18.6	40.58
Tobacco history	0.5-1 ppd	1ppd x 3-4 yr	None
Alcohol history	None	2-4 drinks/month	1 beer weekly
Drug history	None	Cocaine 3x in 2019, cannabis daily, MDMA +LSD in high school	None
Cause of death	N/A	Asphyxiation	N/A
Other	Metastatic colorectal cancer	N/A	Metastatic tumor

Table S5. Primer sequences used for real time RT-PCR of human CYP enzymes.

Gene Name	GeneBank Accession no.	Primer sequence 5' to 3'
CYP1A2	NM_000761	Forward-CTTTGACAAGAACAGTGTCCG
		Reverse-AGTGTCCAGCTCCTTCTGGAT
CYP2B6	NM_000767	Forward-GCACTCCTCACAGGACTCTTG
		Reverse-CCCAGGTGTACCGTGAAGAC
CYP3A4	NM_017460	Forward-CCTTACACATACACACCCTTTGGAAGT
		Reverse-AGCTCAATGCATGATCAGAATCCCCGGTTA
GAPDH	NM_001256799	Forward-GGAGCGAGATCCCTCCAAAAT
		Reverse-GGCTGTTGTCATACTTCTCATGG

Supplementary Figures:

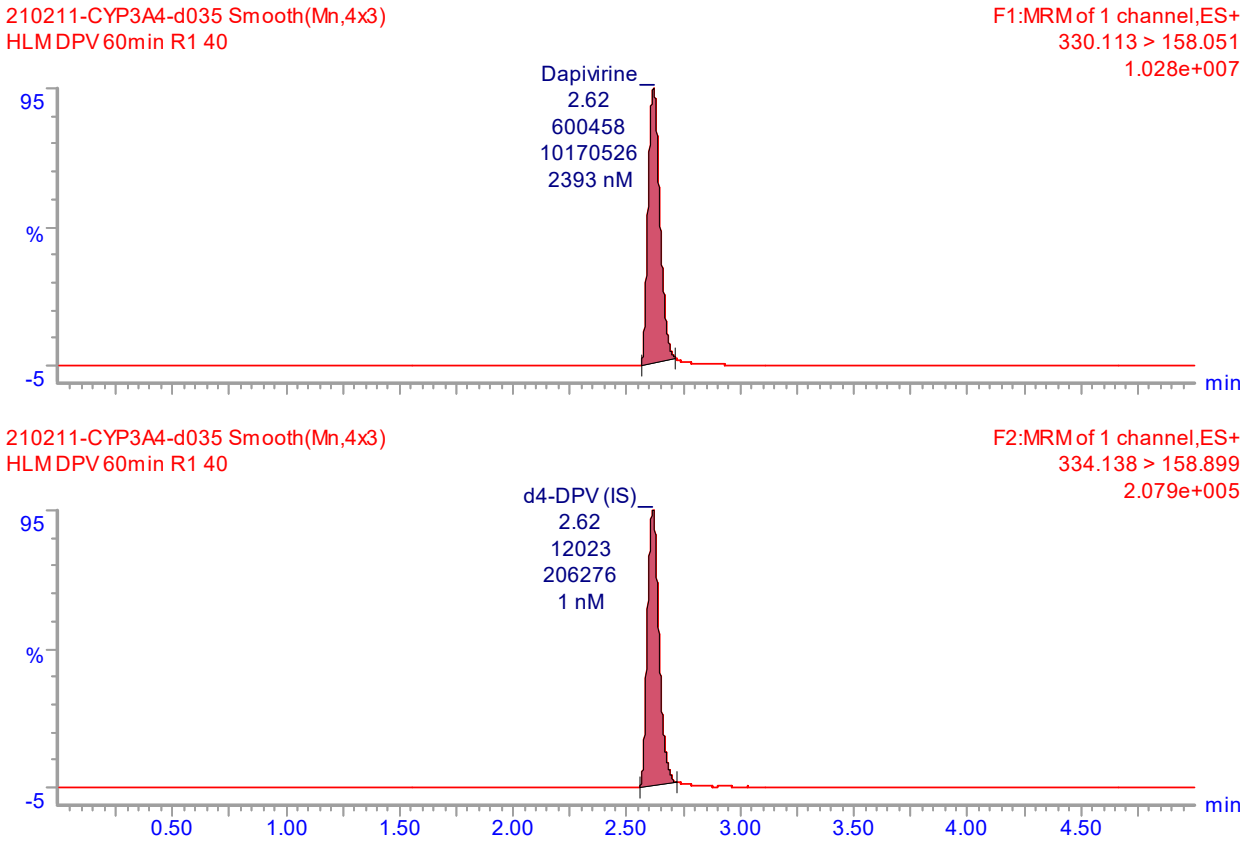

Figure S1. Representative LC-MS/MS chromatograms of DPV and I.S. (d4-DPV.

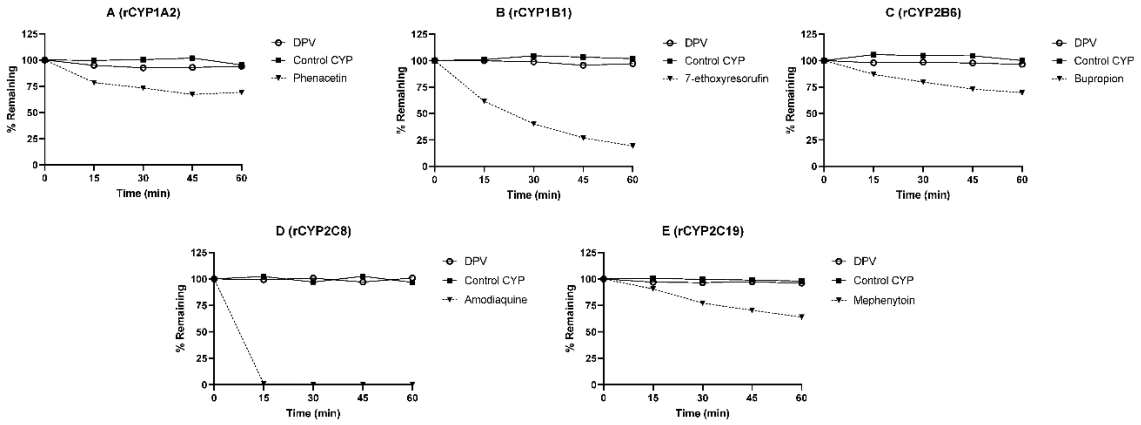

Figure S2. Metabolism of DPV using reaction phenotyping in CYP enzymes. **A)** rCYP1A2, **B)** rCYP1B1, **C)** rCYP2B6, **D)** rCYP2C8, and **E)** rCYP2C19 enzymes. Each test compound/substrate was incubated in a separate incubation mixture. Data are shown as the mean of two biological replicates.

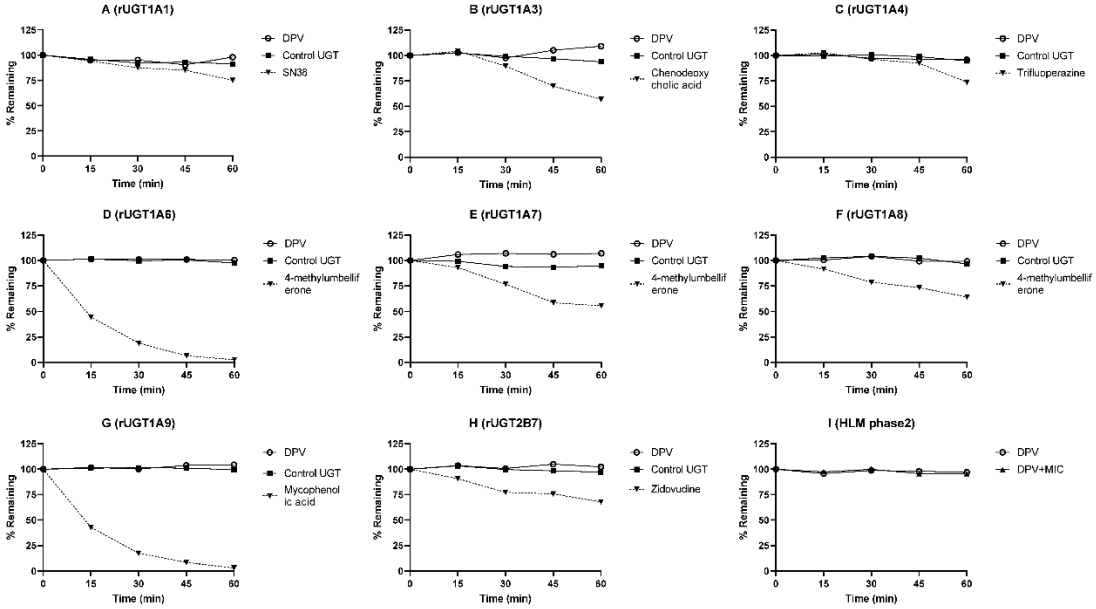

Figure S3. Metabolism of DPV using reaction phenotyping in UGT enzymes. **A)** rUGT1A1, **B)** rUGT1A3, **C)** rUGT1A4, **D)** rUGT1A6, **E)** rUGT1A7, **F)** rUGT1A8, **G)** rUGT1A9, **H)** rUGT2B7 and **I)** HLM (phase 2) enzymes. For HLM (phase 2) enzymes, DPV metabolism was also evaluated in the presence of MIC. Each test compound/substrate was incubated in a separate incubation mixture. Data are shown as the mean of two biological replicates.