Diclofenac and its Acyl Glucuronide: Determination of *In Vivo* Exposure in Human Subjects and Characterization as Human Drug Transporter Substrates *In Vitro*

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Running title: DF and DF-AG transporters in vitro and their in vivo disposition in human

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Table 1. Comparison of transporter activity between pH 5.4/6.0 and pH 7.0.

Uptake was assessed using stably transfected HEK cells that singularly expressed human transporter.

Transporter	Substrate	Transporter activity at pH 5.4 (cell uptake) or 6.0 (vesicle uptake)	Transporter activity at pH7.4
		Mean ± SD (ul/mg/min)	Mean ± SD (ul/mg/min)
OAT1	PAH	85.02±4.38	104.85±11.29
OAT3	E3S	151.03±4.23	240.2±6.4
OAT4	E3S	167.71±20.6	199.79
OATP1B1	E-GLU	28.22±0.89	30.29±3.15
OATP1B3	CCK-8	32.53±1.04	36.7±1.6
OATP2B1	E3S 5.4	50.88±2.86	53.61±5.88
MRP2	E-Glu	186.7±26.7	150.2±20.6
MRP3	E-Glu	533.0±14.1	659.8±23.44
BCRP	MTX	171.7±24.5	60±2.39

Table 2. IC50 or Ki values of CsA against uptake and efflux transporters (resource: www.druginteractioninfo.org)

Transporter	**Probe Substrate**	**Cell System**	**Ki (μM)**	**IC50 (μM)**	**Pubmed Accession #**
BCRP (ABCG2)	estrone-3-sulfate	Membrane Vesicles	6.7		17220244
	methotrexate		7.8		17220244
BSEP (ABCB11)	taurocholic acid	Membrane Vesicles		0.5	21965623
				10	22961681
				1.54	205123
				4.6	24014644
				2	19520776
			9.5		12404239
				0.8	24062352
MRP1 (ABCC1)	calcein AM	Other cells		2.81	23851114
MRP2 (ABCC2)	5(6)- carboxy-2,7-dichlorofluorescein (CDCF)	Membrane Vesicles		5.55	20307659
	cholecystokinin octapeptide (CCK-8)	Membrane Vesicles	24	45.3	15665139
	vinblastine	Caco-2 cells		2.69	12134946
	vinblastine	MDCK-transfected cells		8.11	12134946
MRP3 (ABCC3)	estradiol-17-beta-glucuronide	Membrane Vesicles			24154606
MRP4 (ABCC4)	dehydroepiandrosterone sulfate (DHEAS)	Membrane Vesicles			24154606

NTCP (SLC10A1)	atorvastatin	HEK293-transfected	4.8	4.8	24799396
	rosuvastatin	HeLa-transfected		0.37	16697742
	taurocholic acid	HEK293-transfected		0.8	24062352
		Hepatocytes		2.4	23516635
		Other cells		1.2	23516635
		Other cells		2.1	25740896
OATP1B1 (SLCO1B1)	8-fluorescein-cAMP	CHO-transfected		0.25	20540932
		HEK293-transfected		2.8	23219525
	atorvastatin	HEK293-transfected	0.16		25414411
			0.66	1.5	24799396
			0.82		21861202
			0.014	0.021	20519340
			0.31	0.47	20519340
	bosentan	CHO-transfected		0.3	17496208
	bosentan	HEK293-transfected	0.206		25414411
	bromosulfophthalein (BSP)	HEK293-transfected		0.694	23920221
	bromosulfophthalein (BSP)	MDCK-transfected cells		3.5	16495352
	cerivastatin	Hepatocytes (cryopreserved)	0.28		12538813
	cerivastatin	MDCK-transfected cells	0.238		12538813
	estradiol-17-beta-glucuronide	HEK293-transfected		0.05	17901929
				0.198	23179780
				0.019	23179780
				0.2	16316932
				0.118	23920221
				0.9	22587986
				0.87	23297161
				1.64	23248200
				1.25	23248200
			0.2		15535988
				0.13	23886114
		HeLa-transfected		0.37	12490595
	estrone-3-sulfate	HEK293-transfected		0.732	23920221
					22240838
				1.25	22587986
	fexofenadine (terfenadine carboxylate)	HEK293-transfected	0.0771		25414411
	fluvastatin	HEK293-transfected	0.157		25414411
	glyburide (glibenclamide)	HEK293-transfected	0.102		25414411

	mesalamine (5-ASA)	HEK293-transfected	1		21430235
	nateglinide	HEK293-transfected	0.244		25414411
	phalloidine	HEK293-transfected	0.051	0.5	14530907
	pitavastatin	HEK293-transfected		0.23	23750830
			0.228		25414411
				0.7	22587986
			0.242		16595711
		X. laevis oocytes-injected		2.91	15344842
	pravastatin	HEK293-transfected	0.184		25414411
	repaglinide	HEK293-transfected	0.0857		25414411
	rosuvastatin	HEK293-transfected		0.21	25740896
				0.89	23248200
			0.301		25414411
		HeLa-transfected		0.31	16697742
		X. laevis oocytes-injected		2.2	15289793
	torsemide	HEK293-transfected	0.486		25414411
	valsartan	HEK293-transfected	0.138		25414411
OATP1B3 (SLCO1B3)	8-fluorescein-cAMP	CHO-transfected		0.2	20540932
		HEK293-transfected		2.2	23219525
	amanitin	MDCK-transfected cells		0.3	16495352
	atorvastatin	HEK293-transfected	1.3	3.1	24799396
	bosentan	CHO-transfected		0.8	17496208
	bosentan hydroxy metabolite (Ro 48-5033)	CHO-transfected		0.5	17496208
	bromosulfophthalein (BSP)	MDCK-transfected cells		0.3	16495352
	cholecystokinin octapeptide (CCK-8)	HEK293-transfected		0.8	23297161
		MDCK-transfected cells	1.2	1.8	15665139
	dioscin	HEK293-transfected	1.43		23396419
	estradiol-17-beta-glucuronide	HEK293-transfected		0.162	23179780
				0.032	23179780
				0.057	23886114
	fexofenadine (terfenadine carboxylate)	HEK293-transfected		0.573	18180276
	pitavastatin	HEK293-transfected		0.42	23750830
	rosuvastatin	HEK293-transfected		0.13	25740896
		HeLa-transfected		0.06	16697742
OATP2B1 (SLCO2B1)	rosuvastatin	HeLa-transfected		0.07	16697742

OAT2	Creatinine	HEK293-transfected		11	25904762

Table 3. Clinical relevant drug-drug interaction with diclofenac as a victim (resource: www.druginteractioninfo.org)

Precipitant	Mechanism	Pubmed Accession #	AUC fold changes	Cmax fold changes
cyclosporine	Not provided	8970037	2.05	
diosmin	CYP2C9	17708066	1.60	1.55
fluvastatin	CYP2C9	7586933	1.25	1.14
voriconazole	CYP2C9	18034666	1.76	2.13