Solvo Transporter Symposium (11/14/19)

Long-lasting inhibition of OATPs: Update on the mechanisms and impact

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When determining the IC_{50} or K_i values for OATPs, "pre-incubation for a minimum of 30 min"

OATP1B1 and OATP1B3: The sponsor should conduct studies to determine the inhibition potency (i.e., IC_{50} or K_i) of the investigational drug on the uptake of a known OATP1B1 or OATP1B3 substrate in cells overexpressing the relevant transporter. Because some known OATP1B1/3 inhibitors demonstrate time-dependent inhibition, the sponsor should determine IC_{50} values following pre-incubation with the investigational drug for a minimum of 30 minutes (Amundsen, Christensen, et al. 2010; Gertz, Cartwright, et al. 2013; Izumi, Nozaki, et al. 2015).

(Oct. 2017)

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

(2017)

be utilized. For the determination of K_i value of the investigational drug, typical substrates can be selected from Table 2-1 and their recommended concentration should be sufficiently lower than their K_m value. Also, when calculating the K_i value, preincubation for 30 minutes or more is performed.

Long-lasting inhibition

Short-lasting inhibition

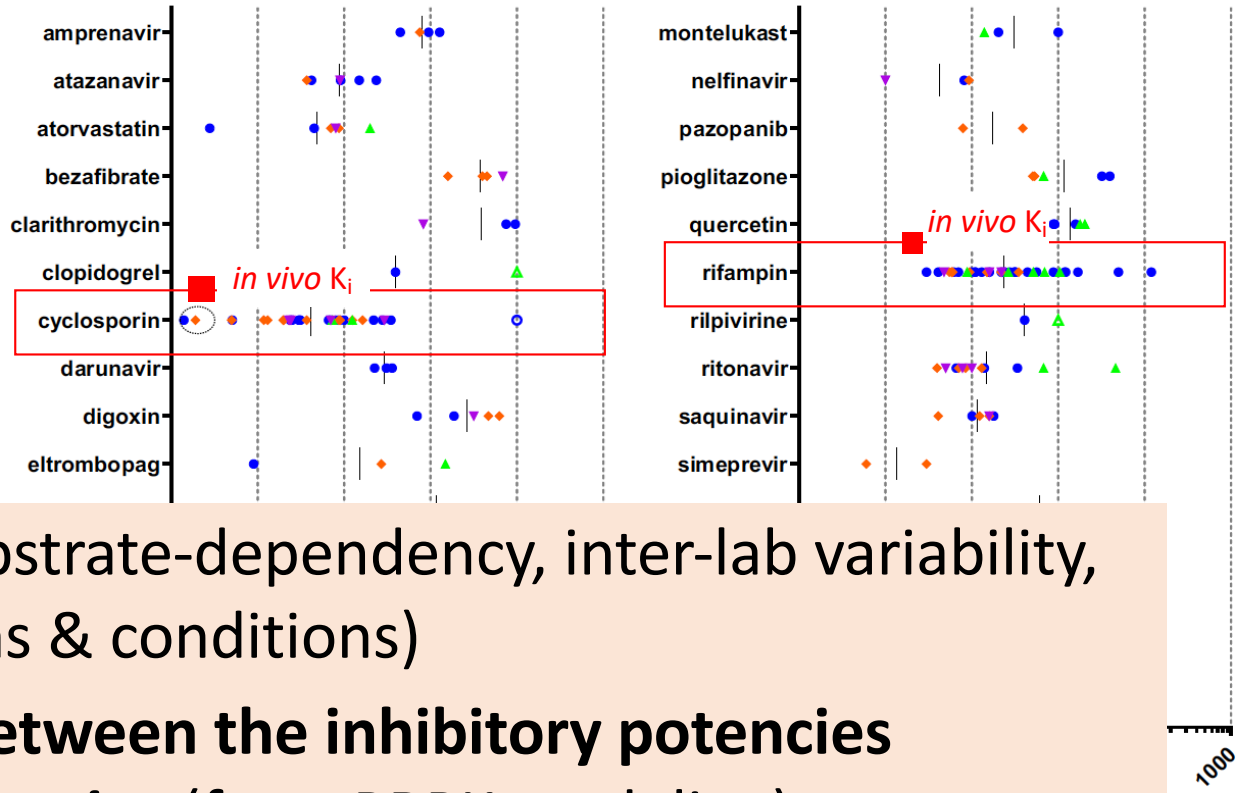
Preincubation-dependent inhibition

Potentiation of transporter inhibition by preincubation (PTIP)

Time-dependent inhibition

$$\frac{[I]}{IC_{50} \text{ or } Ki}$$

Rationale 1



- **Wide variability** (substrate-dependency, inter-lab variability, experimental systems & conditions)
- **Large discrepancy between the inhibitory potencies obtained *in vitro* & *in vivo*** (from PBPK modeling)

***in vitro* K_i or IC_{50} (µM)**

Vaidyanathan et al. J Clin Pharmacol. 2016; PMID: 27385179

Rationale 2

11 substrates, 61 inhibitors of OATP1B1
107 clinical (in vivo) DDI studies

$$R = 1 + I_{u,in,max}/(K_i \text{ or } IC_{50})$$

		R ≥ 1.1?	
		Yes	No
Clinical DDI	Yes	40 (True positive)	**12 (False negative)**
	No	**22 (False positive)**	33 (True negative)

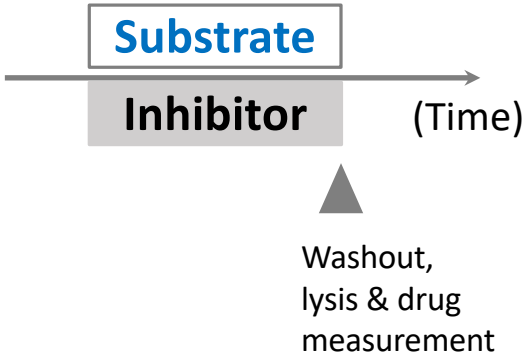
Positive predictive value (=TP/[TP+FP])	**65% (40/62)**
Negative predictive value (=TN/[TN+FN])	**73% (33/45)**

What can we do to reduce false (+/−) predictions?

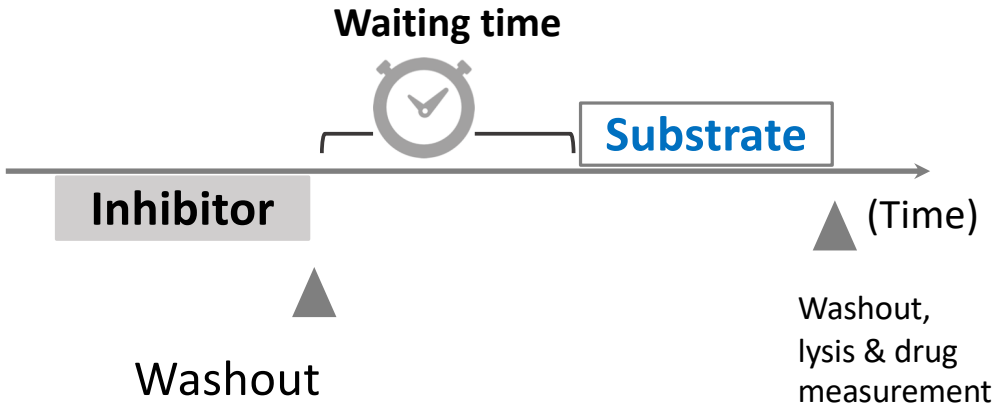
Vaidyanathan et al. J Clin Pharmacol. 2016; PMID: 27385179

How can we obtain *in vivo* relevant IC_{50} or Ki?

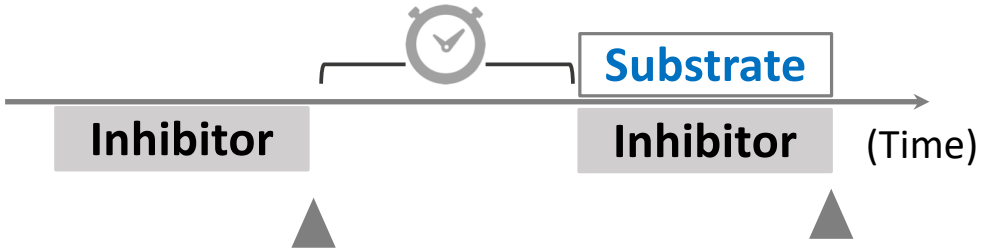
Co-incubation



Pre-incubation



Pre- & co-incubation



- ✓ Preincubation-dependent inhibition
- ✓ Time-dependent inhibition
- ✓ Long-lasting inhibition
- ✓ Short-lasting inhibition

List (as of 2017)

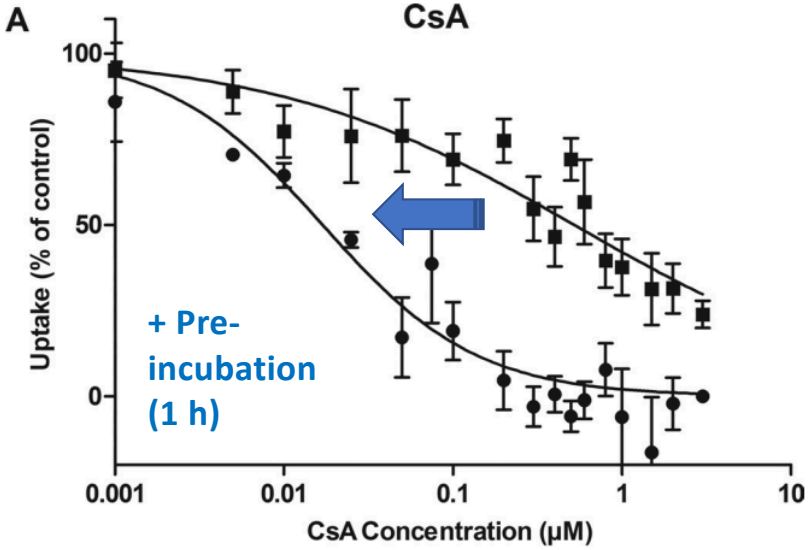
Transporters	Inhibitors	
	Preincubation time-dependent enhancement effect of transporter inhibition	
	Positive (+)	Negative (−)[a]
OATP1B1	CsA[b] (and AM1) Simeprevir Asunaprevir Ritonavir (weak)[b] Gemfibrozil (weak)	Tacrolimus Saquinavir[b] Rifampicin Rifamycin SV Sildenafil Clarithromycin Erythromycin Telmisartan Glibenclamide Ketoconazole
OATP1B3	CsA[b] (and AM1) Simeprevir Asunaprevir	
OATP2B1	Apple juice[b] Orange juice[b]	
OAT1	Chrysophanol Physcion	Probenecid Rhein Emodin Aloe-emodin
OAT3	Chrysophanol Physcion Emodin Aloe-emodin	Probenecid Rhein

Shitara & Sugiyama Pharmacol Ther. 2017; PMID: 28249706

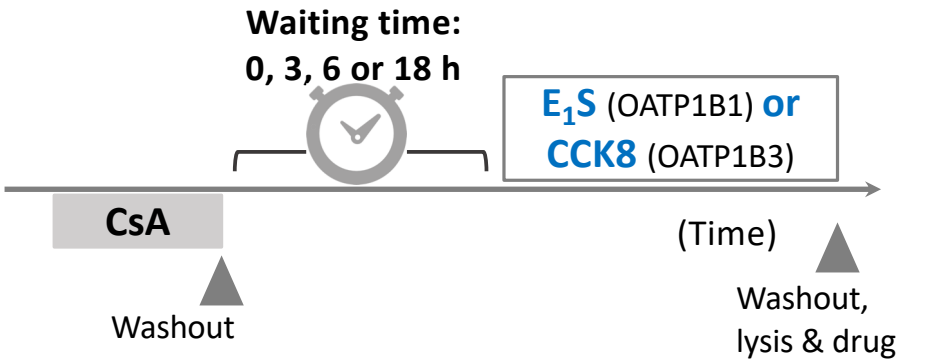
List expands... (as of Nov. 2019)

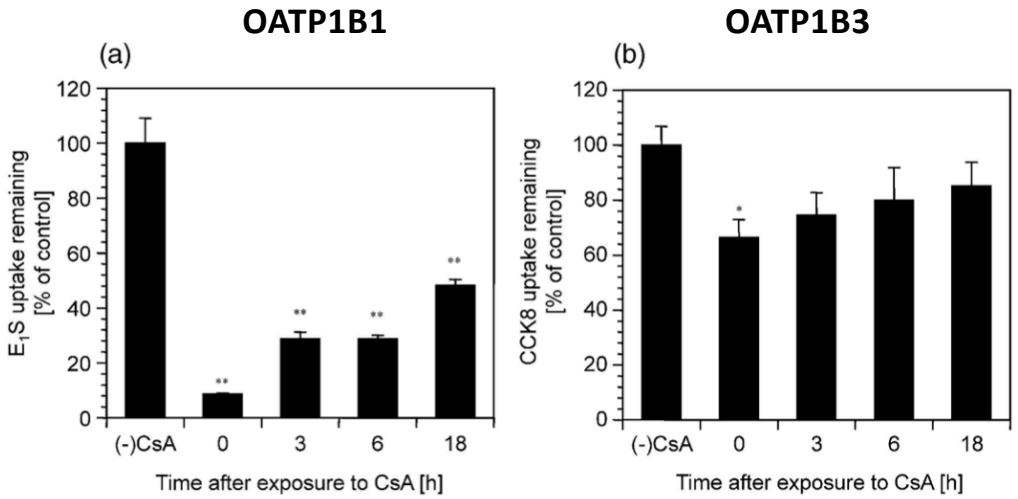
Transporters	Inhibitors	references
OAT1/3	anthraquinones	Ma et al. 2015
OATP1B1/1B3	**rifampicin, dasatinib**	Pahwa et al. 2017
OCT2	**crizotinib**	Arakawa et al. 2017; Omote et al. 2018
OATP1B3	pentacyclic triterpenoids (betulinic acid, ursolic acid, oleanolic acid)	Oh et al. 2018
OATP1B1/1B3	everolimus, sirolimus	Farasyn et al. 2019
OCT1	CsA	Panfen et al. 2019
OATP1B1	**pazopanib**	Taguchi et al. 2019
OATP1B1/3	**venetoclax**, saquinavir	Tátrai et al. 2019
OCT1/2	**ledipasvir**, daclatasvir, **vandetanib**, cetirizine, isavuconazole	
MATE2-K	vandetanib	

Early Examples (CsA)



(Amundsen et al. 2010; PMID 20519340)

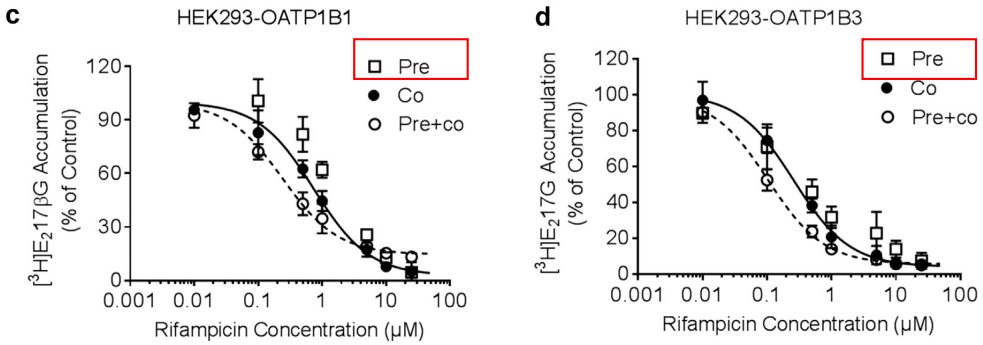




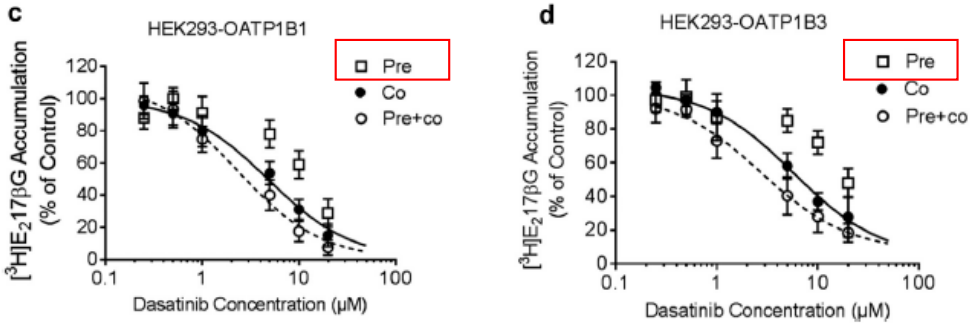
(Shitara et al. 2012; PMID 20519340)

More Examples (Rif, TKIs)

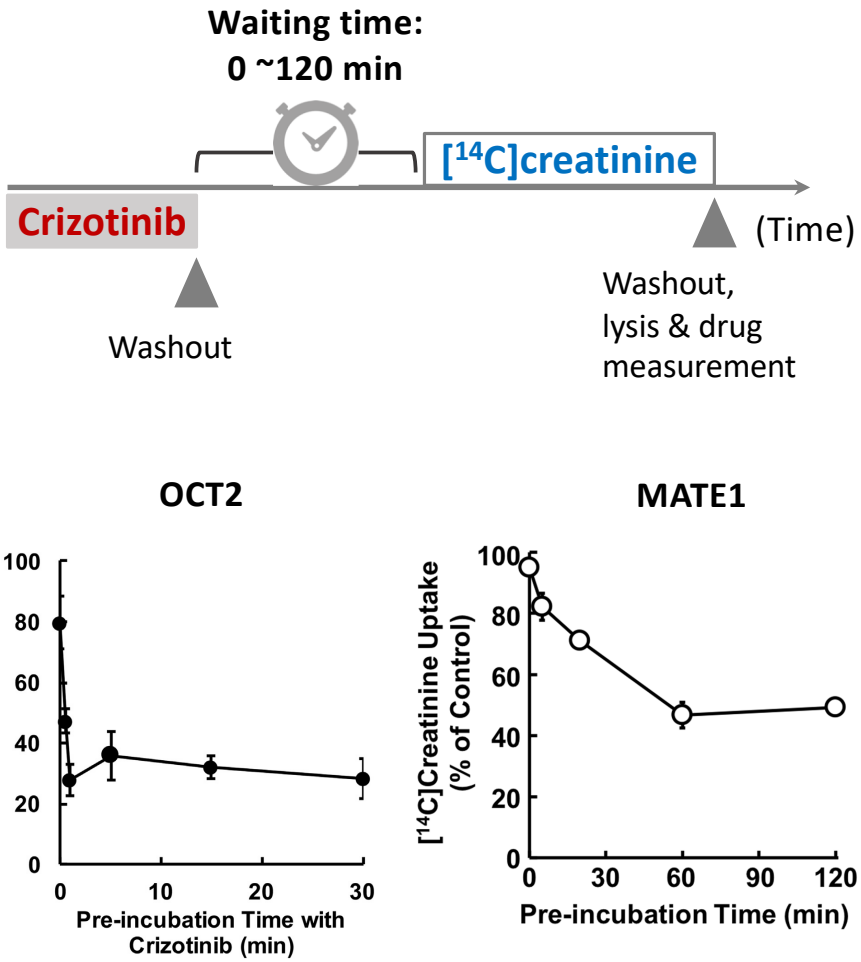
Rifampicin (1 h pre-incubation, no waiting time @4˚C)



Dasatinib (1 h pre-incubation, no waiting time @4˚C)

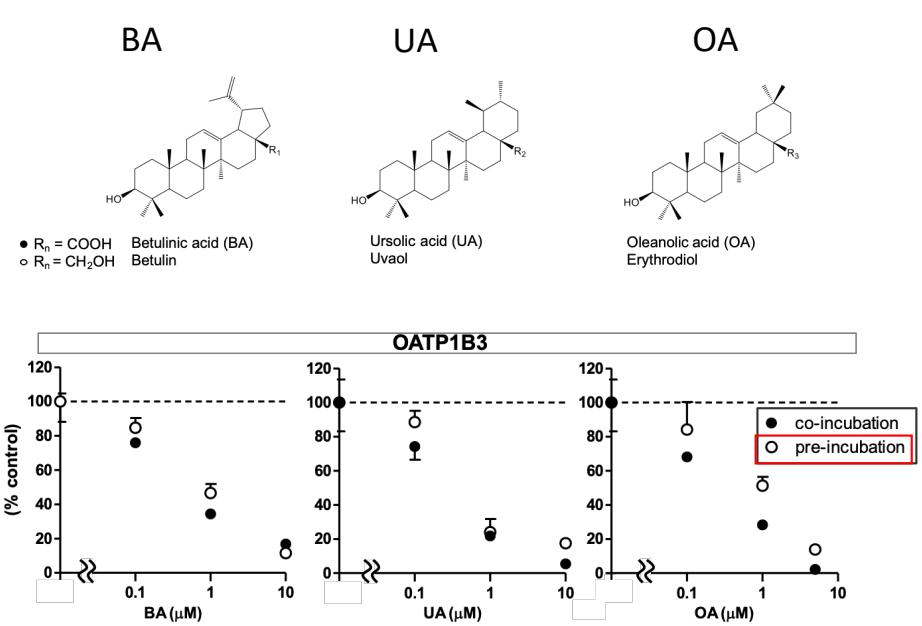


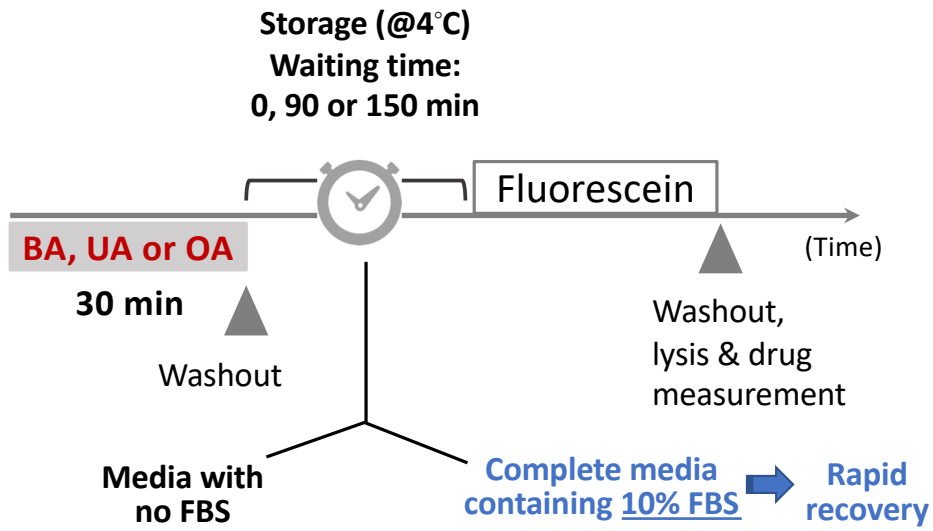
(Pahwa et al. 2017)



(Arakawa et al. 2017; Omote et al. 2018)

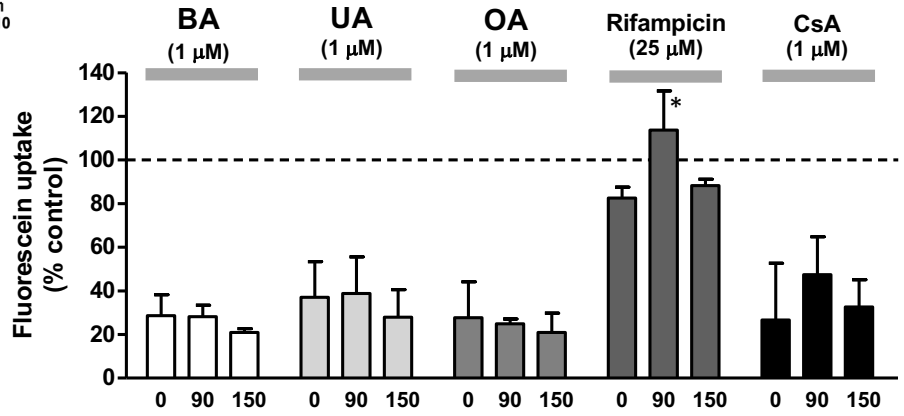
More Examples (Pentacyclic Triterpenoids)





Experimental conditions matters in teasing out "pre-incubation" effects (consideration of non-specific binding, protein binding, etc.)

(Oh et al. J Pharm Sci 2018)



Mechanism 1: Non-specific binding??

- The time required for maximum enhancement by pre-incubation: compound-dependent
- Compounds tend to be highly lipophilic and protein bound

	cLogP	f_u (plasma)
CsA	14.0	0.015
simeprevir	4.8	0.001
asunaprevir	3.1	<0.01
venetoclax	10.0	0.000013
ledipasvir	6.7	<0.002
daclatasvir	4.7	<0.01
pazopanib	3.6	<0.01
daclatasvir	4.7	0.01
betulinic acid	6.6	0.0001
crizotinib	3.7	0.09

A Systematic In Vitro Investigation of the Inhibitor Preincubation Effect on Multiple Classes of Clinically Relevant Transporters

Péter Tátrai, Patrick Schweigler, Birk Poller, Norbert Domange, Roelof de Wilde, Imad Hanna, Zsuzsanna Gáborik, and Felix Huth

Drug Metab Dispos. 2019; PMID: 31068368

Plasticware precoated against non-specific binding (NSB) using 20% (v/v) FBS, 2% (w/v) BSA

PTIP: Potentiation of transporter inhibition by pre-incubation for **3 h**

Inhibitor	PTIP (Fold Potentiation)			
	NSB Block −	NSB Block +	NSB Block −	NSB Block +
	OATP1B1		OATP1B3	
→ Venetoclax	**203**	**>258**	**>13.2**	**>8.70**
Cyclosporin A	**5.88**	**6.78**	**3.75**	**3.02**
Saquinavir	**3.17**	**3.54**	**4.60**	**3.78**
Atorvastatin	1.88		2.26	
Rifampicin	1.47		1.72	
Gemfibrozil	0.976		0.672	
	OAT1		OAT3	
Benzbromarone	1.82		**4.96**	1.51
Furosemide	1.44		0.723	
Valsartan	1.40		1.58	
Probenecid	1.37		1.58	
Diclofenac	1.25		0.428	
Bumetanide	1.19		1.32	
Gemfibrozil	0.514		1.21	
Rifampicin	N/D		N/D	
Saquinavir	N/D		N/D	
Pravastatin	N/D		N/D	

Inhibitor	PTIP (Fold Potentiation)			
	NSB Block −	NSB Block +	NSB Block −	NSB Block +
	OCT1		OCT2	
→ Ledipasvir	**>594**	**>255**	**>4.04**	**>8.73**
Irinotecan	**17.3**	**5.43**	2.12	
Saquinavir	**7.81**	**4.09**	N/D	
→ Daclatasvir	**3.42**	**5.65**	**156**	**34.2**
Verapamil	**3.36**	**3.06**	1.86	
Vandetanib	**3.14**	**2.69**	**5.36**	**4.19**
Cetirizine	**3.12**	1.78	**3.35**	1.55
Isavuconazole	**3.00**	**2.98**	**5.52**	**13.2**
Cimetidine	1.71		N/D	
Amisulpride	1.61		1.18	
Ranolazine	1.35		1.08	
Trimethoprim	1.35		1.28	
Abacavir	0.892		N/D	
Dolutegravir	N/D		**6.20**	**11.3**
	MATE1		MATE2-K	
Pyrimethamine	1.88		2.09	
Vandetanib	1.81		**2.54**	**3.00**
Trimethoprim	1.75		1.07	
Ondansetron	1.57		1.04	
Isavuconazole	1.53		2.46	1.38
Cimetidine	1.27		0.747	
Famotidine	1.12		1.73	
Ranitidine	0.668		0.438	

Venetoclax

VENCLEXTA® venetoclax tablets 10mg, 50mg, 100mg

- Oral selective BCL-2 inhibitor
- Initial FDA approval (2016) for the treatment of acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), or small lymphocytic lymphoma (SLL)

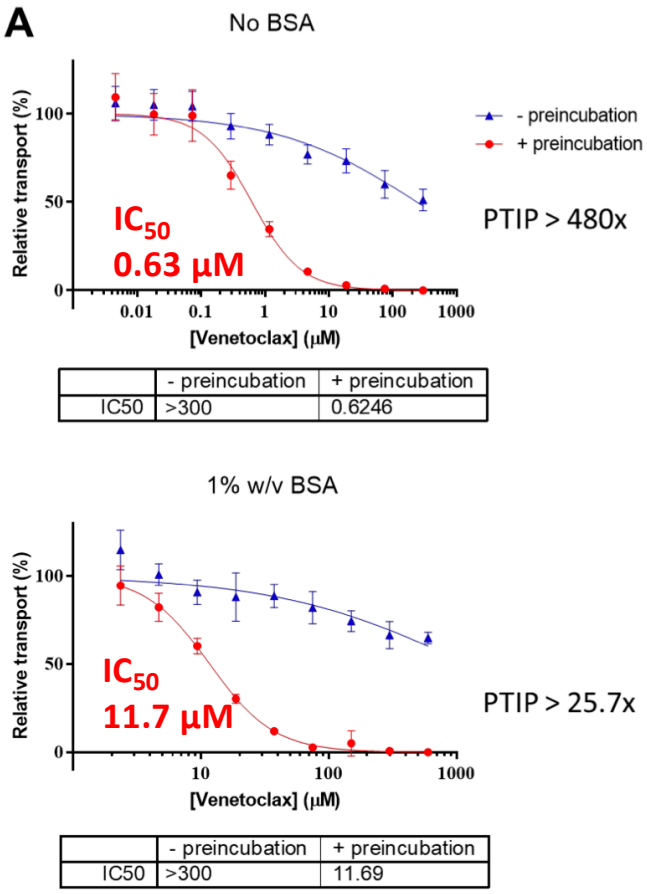
IC50 values of venetoclax

Protein Inhibited	Venetoclax [μM]
P-gp	30.0 ± 3.7
BCRP	19.6 ± 7.3
OATP1B1	47.8 ± 10.1
OATP1B3	26.0 ± 9.6
CYP1A2	no inhibition
CYP2B6	activation
CYP2C19	14.21 ± 1.0
CYP2D6	no inhibition
CYP3A4	7.2 ± 3.2

Tested using 8-FcA as a probe substrate (10 min incubation) in HEK-OATP1B1 & HEK-OATP1B3

(Weiss et al 2016; PMID: 26927160)

OATP1B1



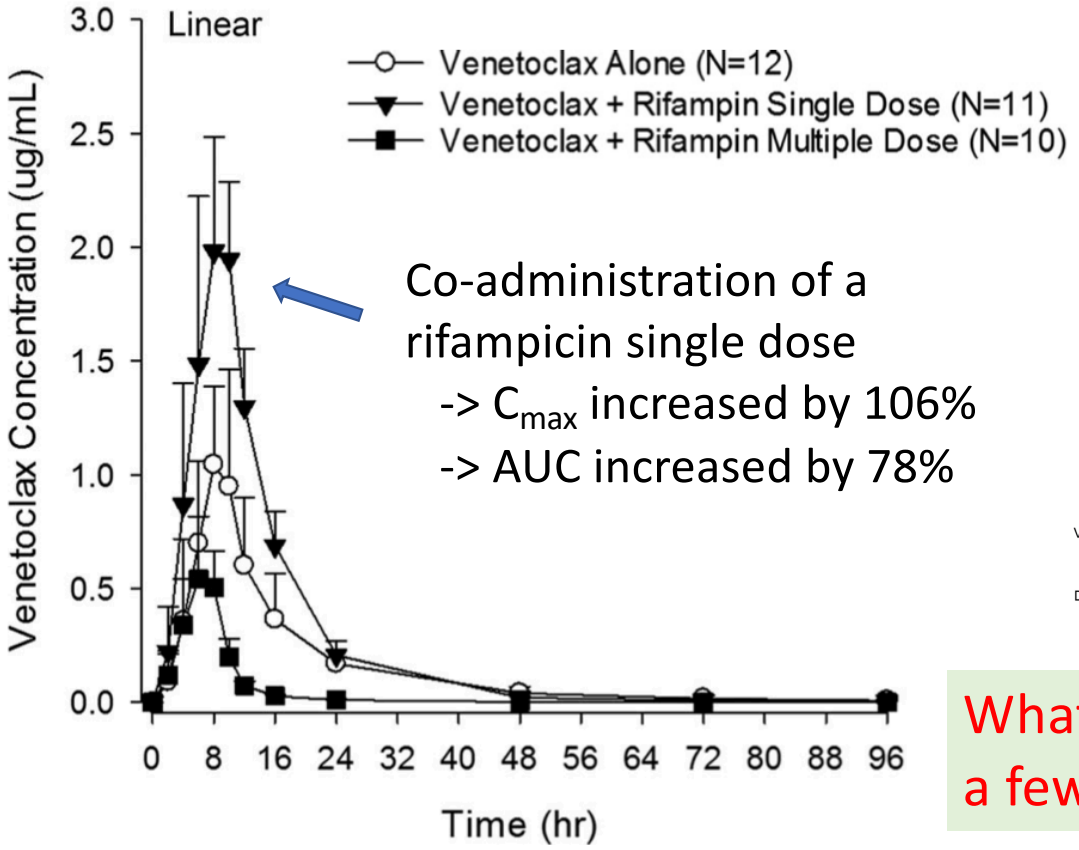
	- preincubation	+ preincubation
IC50	>300	0.6246

	- preincubation	+ preincubation
IC50	>300	11.69

(Tátrai et al. 2019; PMID: 31068368)

Venetoclax

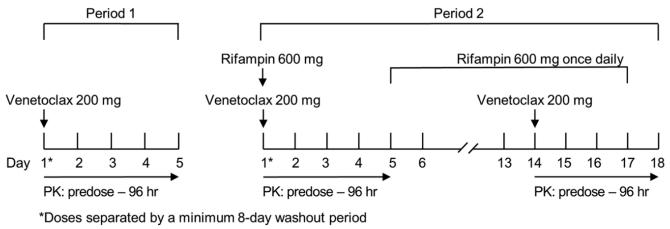
Clinical DDI??



Co-administration of a rifampicin single dose
-> C_{max} increased by 106%
-> AUC increased by 78%

possible DDI mechanisms??

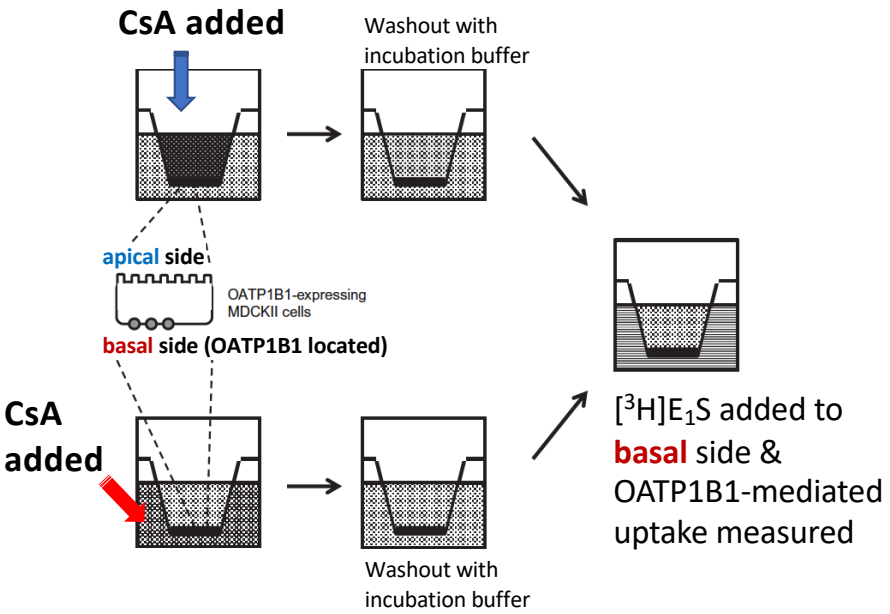
2016	**2019**
likely via Pgp inhibition	**OATP1B inhibition??**

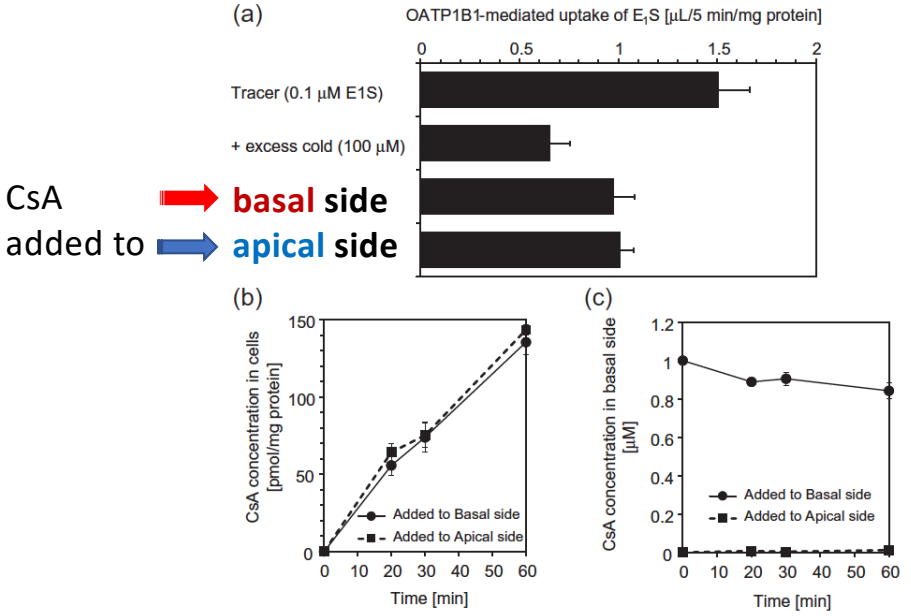


What if venetoclax was administered a few hrs before rifampin dosing?

(Agarwal et al. 2016; PMID 26953185)

Mechanism 2: trans-inhibition?

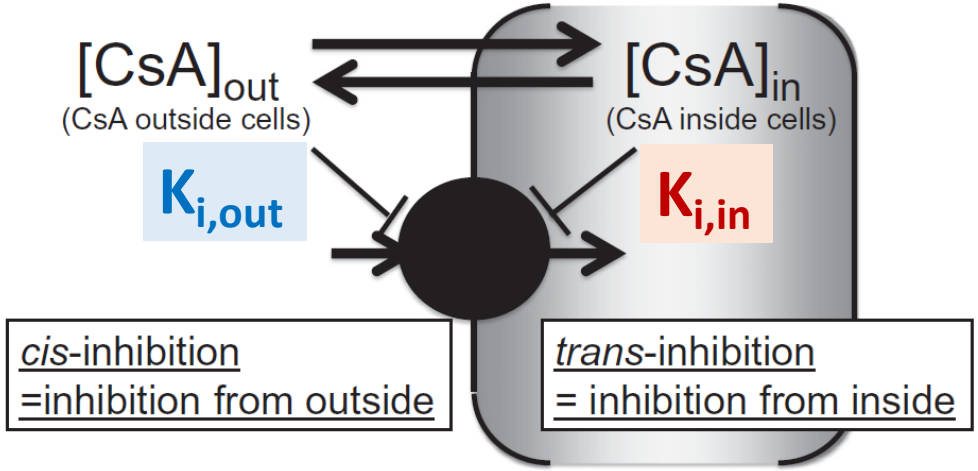




Long-lasting inhibition of OATP1B1 by CsA could be driven by CsA inside the cells.

Shitara & Sugiyama Pharmacol Ther. 2017; PMID: 28249706

Mechanism 2: *trans*-inhibition?

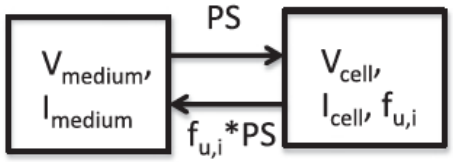


$$CL_{uptake\ (+CsA)} = \frac{V_{max}/\left(1 + f_{u,H} \cdot \frac{[CsA]_{in}}{K_{i,in}}\right)}{K_m \cdot \left(1 + f_{u,B} \cdot \frac{[CsA]_{out}}{K_{i,out}}\right) + S}$$

trans-inhibition (non-competitive)

cis-inhibition (competitive)

Shitara & Sugiyama Pharmacol Ther. 2017; PMID: 28249706



$$CL_{uptake\ (+CsA)} = \frac{V_{max}/(1 + f_{u,H} \cdot \frac{[CsA]_{in}}{K_{i,in}})}{K_m \cdot \left(1 + f_{u,B} \cdot \frac{[CsA]_{out}}{K_{i,out}}\right) + S}$$

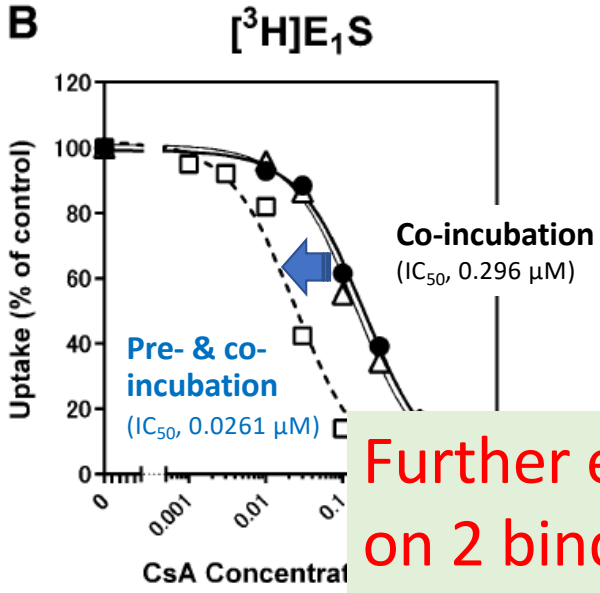
$$V_{cell} \cdot \frac{dI_{cell}}{dt} = -f_{u,i} \cdot PS \cdot I_{cell} + PS \cdot I_{medium}, \quad (1)$$

$$V_{medium} \cdot \frac{dI_{medium}}{dt} = -PS \cdot I_{medium} + f_{u,i} \cdot PS \cdot I_{cell}, \quad (2)$$

$$\frac{dX_{cell}}{dt} = \frac{V_{max}/(1 + f_{u,i} \cdot \frac{I_{cell}}{K_{i,in}})}{K_m \cdot \left(1 + \frac{I_{medium}}{K_{i,out}}\right) + C_{medium}} C_{medium} \quad (3)$$

>>

$$\frac{dX_{cell}}{dt} = \frac{V_{max}/(1 + f_{u,i} \cdot \frac{I_{cell}}{K_{i,in}})}{K_m \cdot \left(1 + \frac{I_{medium}}{K_{i,out}}\right)} C_{medium} \quad (4)$$



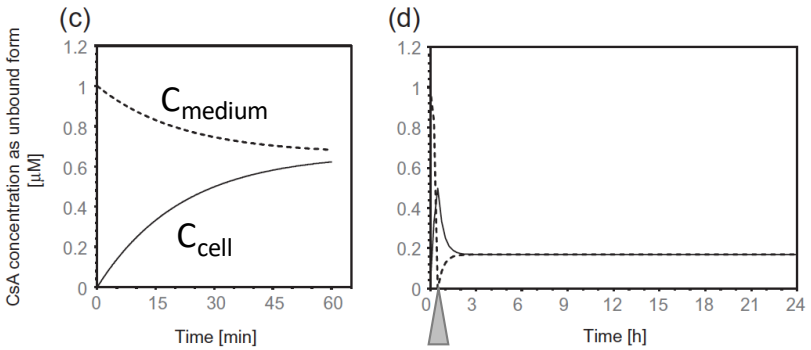
Further evidence on 2 binding sites??

By fitting to the observed data,

$K_{i,in}$	0.0113 µM
$K_{i,out}$	0.530 µM

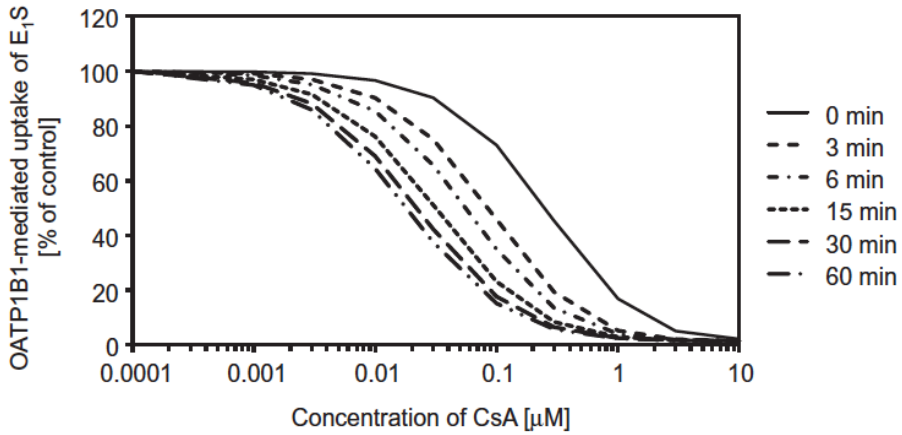
Shitara & Sugiyama Pharmacol Ther. 2017; PMID: 28249706

Simulation of CsA concentrations: in cells vs medium



Wash & change to CsA-free medium

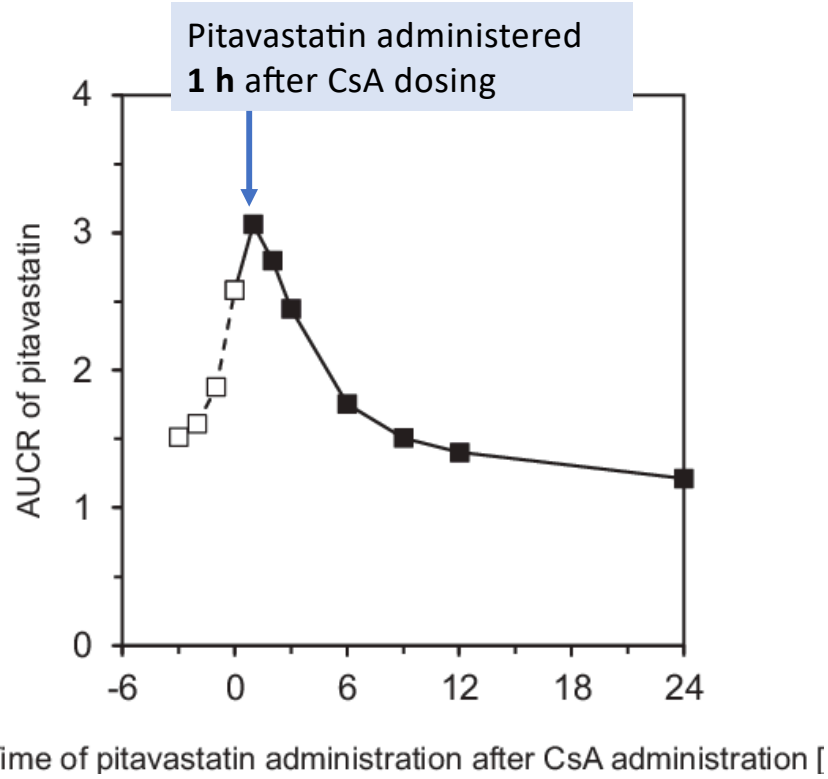
Simulation of pre-incubation effects on the OATP1B1 inhibition by CsA



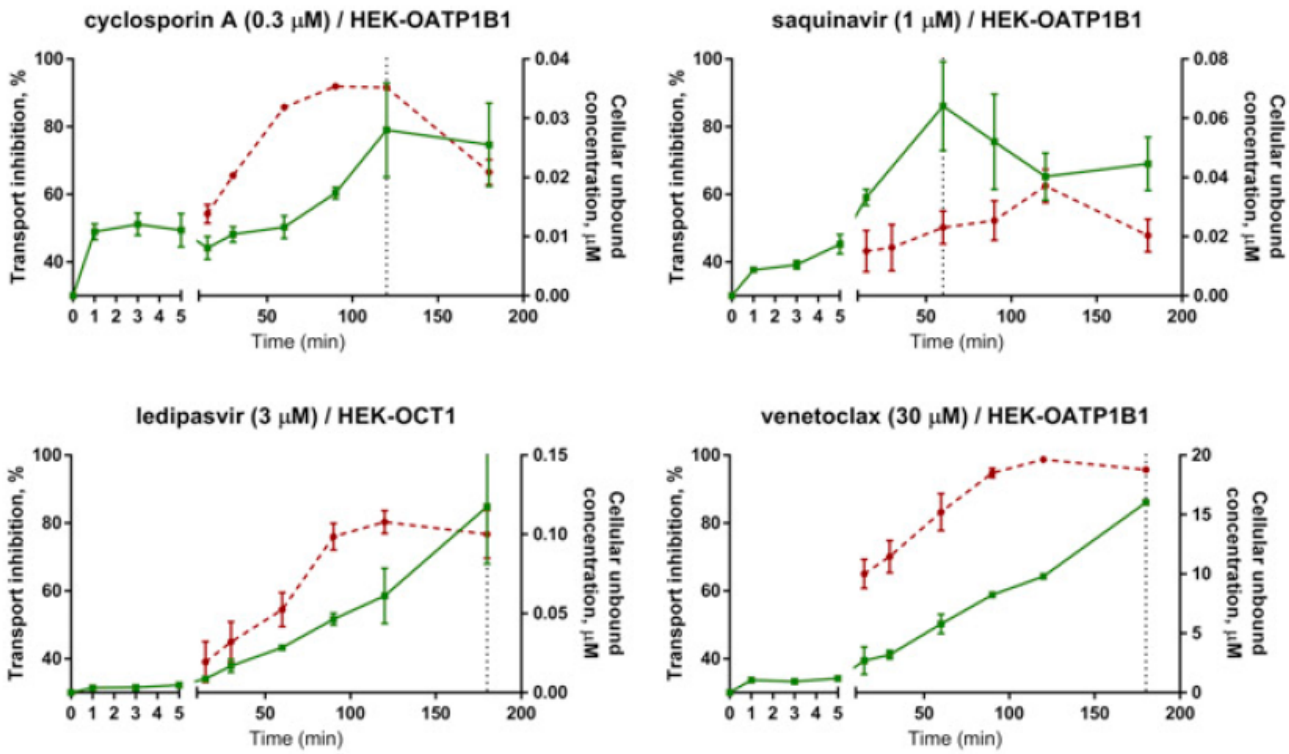
Shitara & Sugiyama Pharmacol Ther. 2017; PMID: 28249706

Prediction of pitavastatin AUC changes by CsA coadministration at different time intervals

(via PBPK modeling considering *trans*-inhibition)



Transport inhibition (%) **Cellular drug conc (µM)**

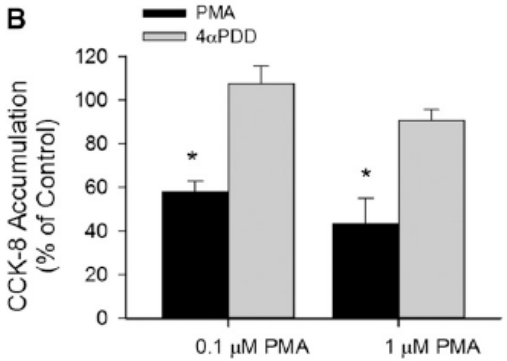


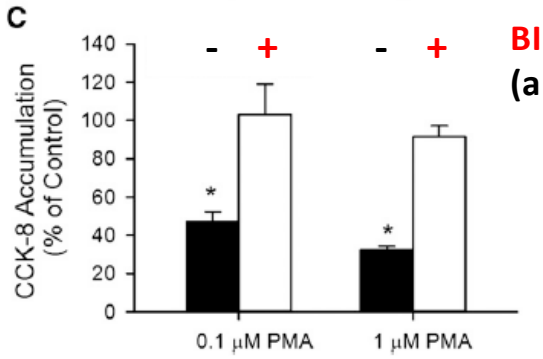
Tátrai et al. Drug Metab Dispos. 2019; PMID: 31068368

These profiles support that **the potentiation of the transport inhibition by pre-incubation is likely driven by drug concentrations inside cells.**

Mechanism 3: Internalization, post-translational regulation??

OATP1B3



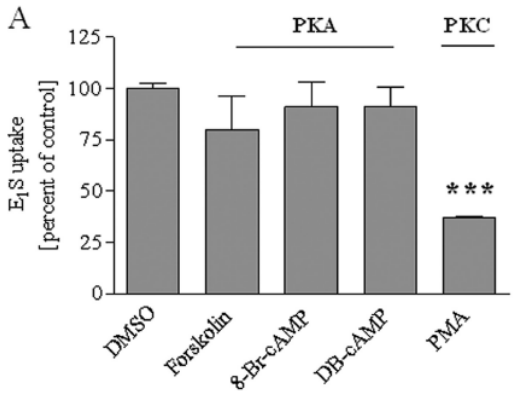


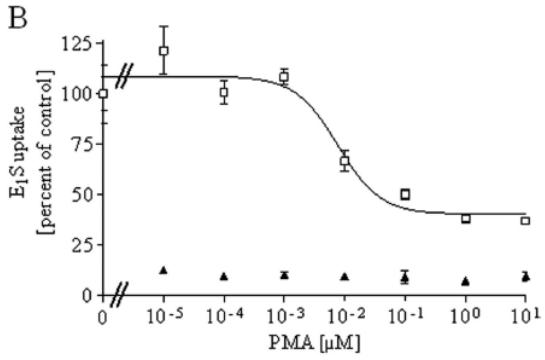
BIM-I
(a PKC inhibitor)

Powell et al. Drug Metab Dispos. 2014; PMID: 25200870

PMA: Protein kinase C (PKC) activator

OATP2B1

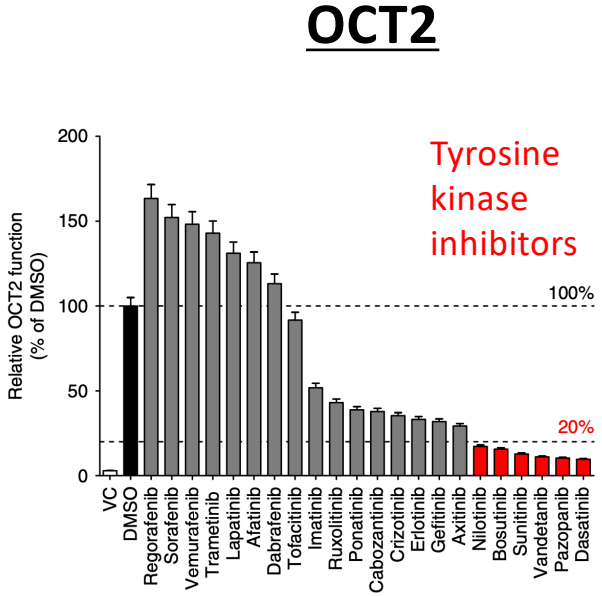


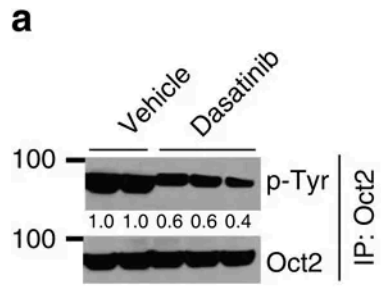


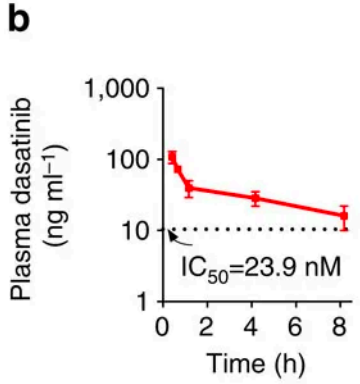
PKC activation
-> ⇧ phosphorylation of OATP2B1,
⇩ OATP2B1 transport activity (⇩ V_{max})

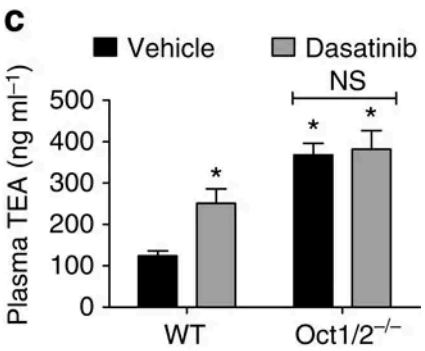
Kock et al. J Biol Chem. 2010; PMID: 20159975

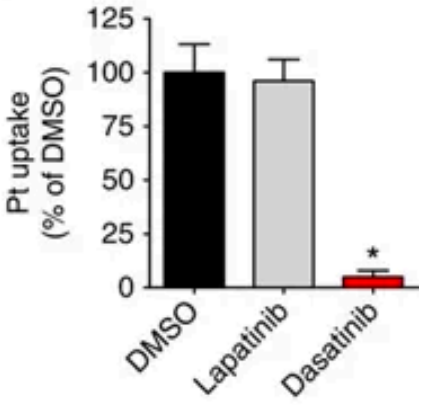
Mechanism 3: Internalization, post-translational regulation??







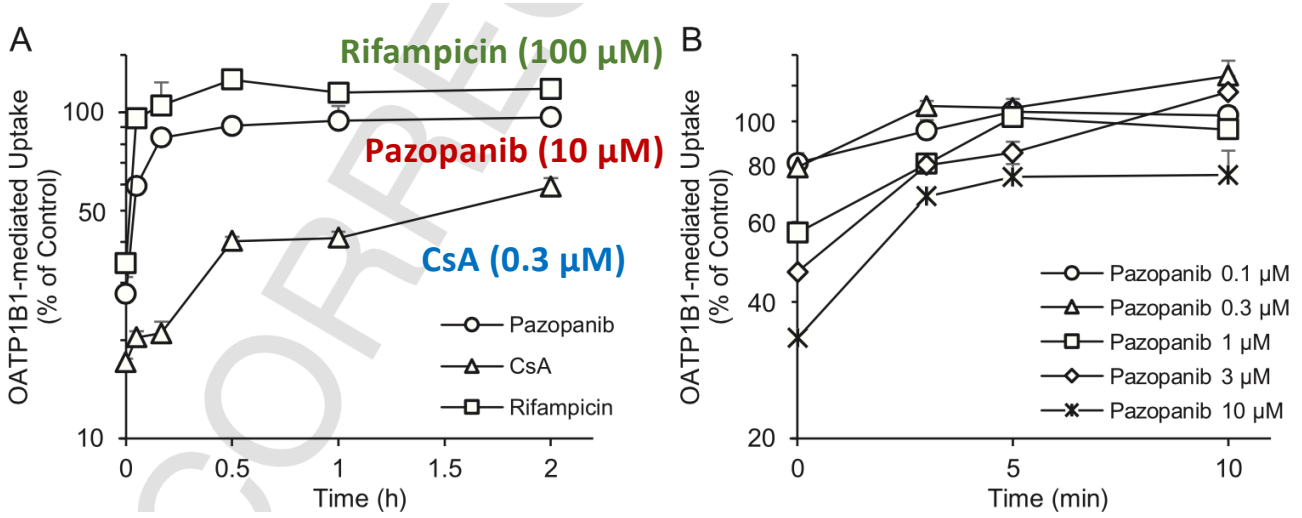




OCT2 activity regulated by p-Tyr switch (mediated by the Src family kinase Yes1); TKIs can reduce oxaliplatin uptake & toxicity *in vivo*

Sprowl et al. Nat Commun. 2016; PMID: 26979622

Recovery time



"Short-lasting inhibition"

$k_{recovery}$ values after preincubation with pazopanib, CsA, and rifampicin.

Inhibitors	Concentration (μM)	$k_{recovery}$ (h^{-1})[a]
Pazopanib	0.1	>3.31
	0.3	>6.26
	1	6.99
	3	7.16
	10	5.62–9.46[b]
CsA	0.3	0.546
Rifampicin	100	>20.4

[a] The $k_{recovery}$ was calculated by replotting the data shown in Fig. 3 into sigma-minus plot in logarithmic scale, followed by linear regression analysis.
[b] The values of 5.62 and 9.46 were obtained from Fig. 3A and B, respectively.

The reduced OATP1B1 activity by pazopanib was much more rapidly recovered than CsA. (<0.5 h *vs* a few hours)

Differences in mechanisms??

Taguchi et al. DMPK 2019;
https://doi.org/10.1016/j.dmpk.2019.08.001

Coming ahead~

- List will expand as more compounds will be tested with pre-incubation (the results will likely vary in terms of the extent/recovery time of preincubation-dependent inhibition; key molecular features/descriptors may emerge)
- Better mechanistic understanding (further evidence on "*trans*-inhibition"; binding equilibrium)

List (as of 2017)

Transporters	Inhibitors	
	Preincubation time-dependent enhancement effect of transporter inhibition	
	Positive (+)	Negative (−)[a]
OATP1B1	CsA[b] (and AM1) Simeprevir Asunaprevir Ritonavir (weak)[b] Gemfibrozil (weak)	Tacrolimus Saquinavir[b] Rifampicin Rifamycin SV Sildenafil Clarithromycin Erythromycin Telmisartan Glibenclamide Ketoconazole
OATP1B3	CsA[b] (and AM1) Simeprevir Asunaprevir	
OATP2B1	Apple juice[b] Orange juice[b]	
OAT1	Chrysophanol Physcion	Probenecid Rhein Emodin Aloe-emodin
OAT3	Chrysophanol Physcion Emodin Aloe-emodin	Probenecid Rhein

Shitara & Sugiyama Pharmacol Ther. 2017; PMID: 28249706

Transporters	Inhibitors	references
OAT1/3	anthraquinones	Ma et al. 2015
OATP1B1/1B3	**rifampicin, dasatinib**	Pahwa et al. 2017
OCT2	**crizotinib**	Arakawa et al. 2017; Omote et al. 2018
OATP1B3	pentacyclic triterpenoids (betulinic acid, ursolic acid, oleanolic acid)	Oh et al. 2018
OATP1B1/1B3	everolimus, sirolimus	Farasyn et al. 2019
OCT1	CsA	Panfen et al. 2019
OATP1B1	**pazopanib**	Taguchi et al. 2019
OATP1B1/3	**venetoclax**, saquinavir	Tátrai et al. 2019
OCT1/2	**ledipasvir**, daclatasvir, **vandetanib**, cetirizine, isavuconazole	
MATE2-K	vandetanib	

Coming ahead~

- Less variability in assessing the transport inhibition potency (K_i or IC_{50} values); **refinement of experimental conditions for pre-incubation studies (pre-incubation times; control of non-specific binding; assay duration)**
- More mechanism-based PBPK modeling; estimation of intracellular unbound drug concentrations; validation & implementation of endogenous biomarkers ⇨ **more accurate DDI prediction (reducing false negative/positive predictions)**

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