



# Active Intestinal Drug Uptake: The Double-Edged Sword Transporters

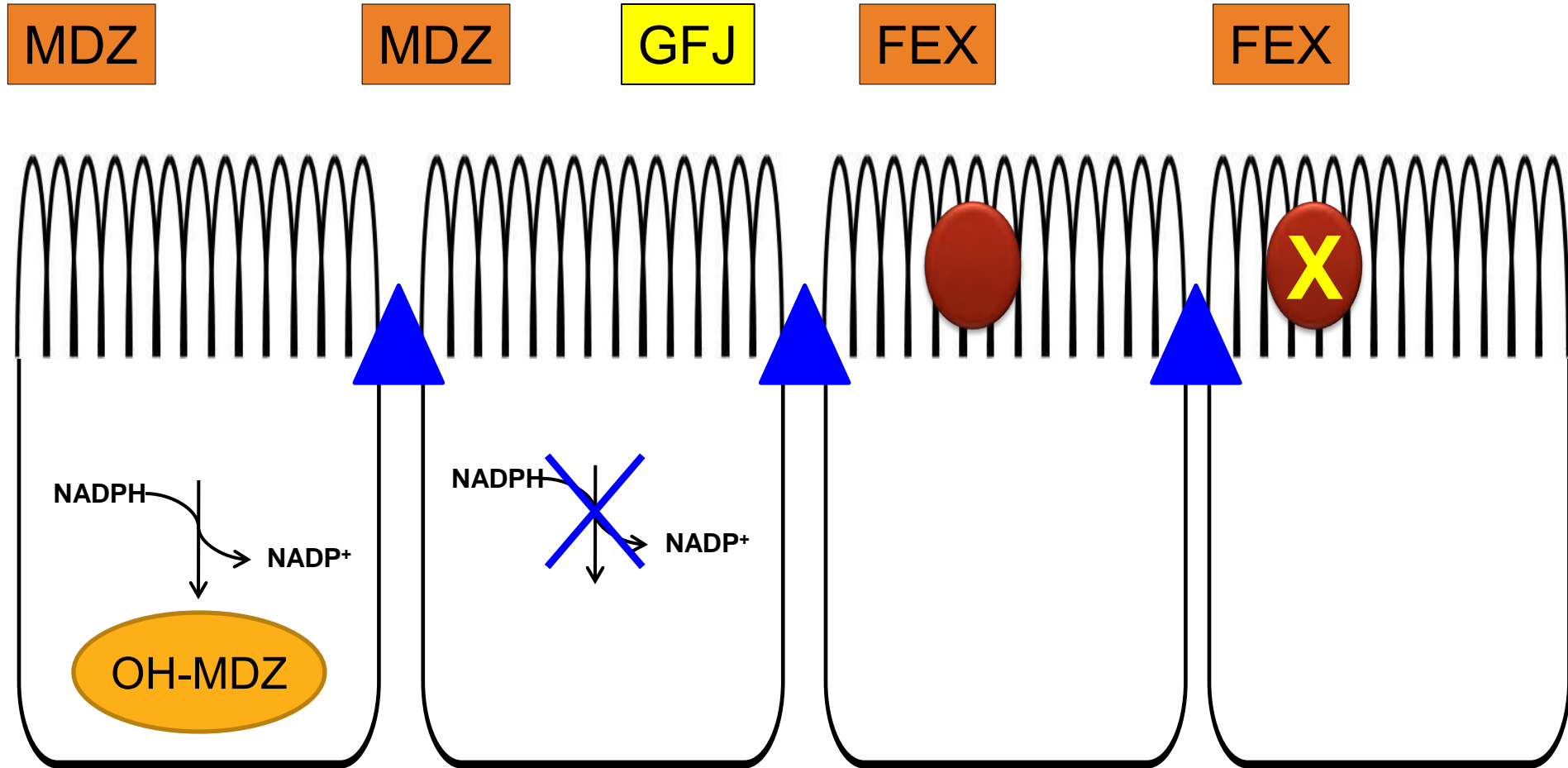
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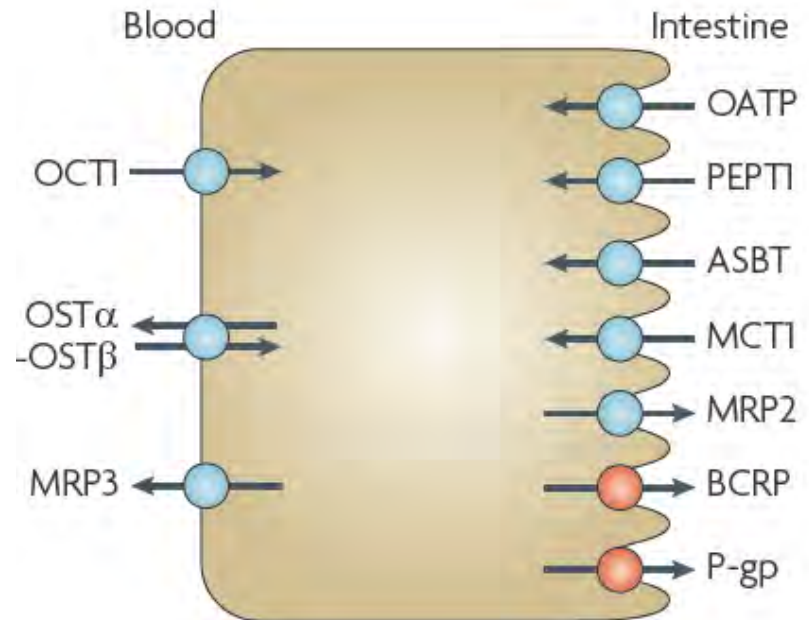
# Grapefruit juice: The multi-tasking inhibitor



**MDZ:** midazolam  
**FEX:** fexofenadine  
 **NOVARTIS**

# Candidate uptake drug transporters in the gastrointestinal tract that may be involved in drug absorption (and interactions)

- Peptide transporters
- Apical Sodium-dependent Bile Acid Transporter (ASBT)
- Organic Anion Transporting Polypeptides (OATP2B1 and OATP1A2)
- Mono-carboxylate transporters (MCTs)



Adapted from: *Giacomini et al., (2010) Nat Rev Drug Discov. 9(3):215-36*

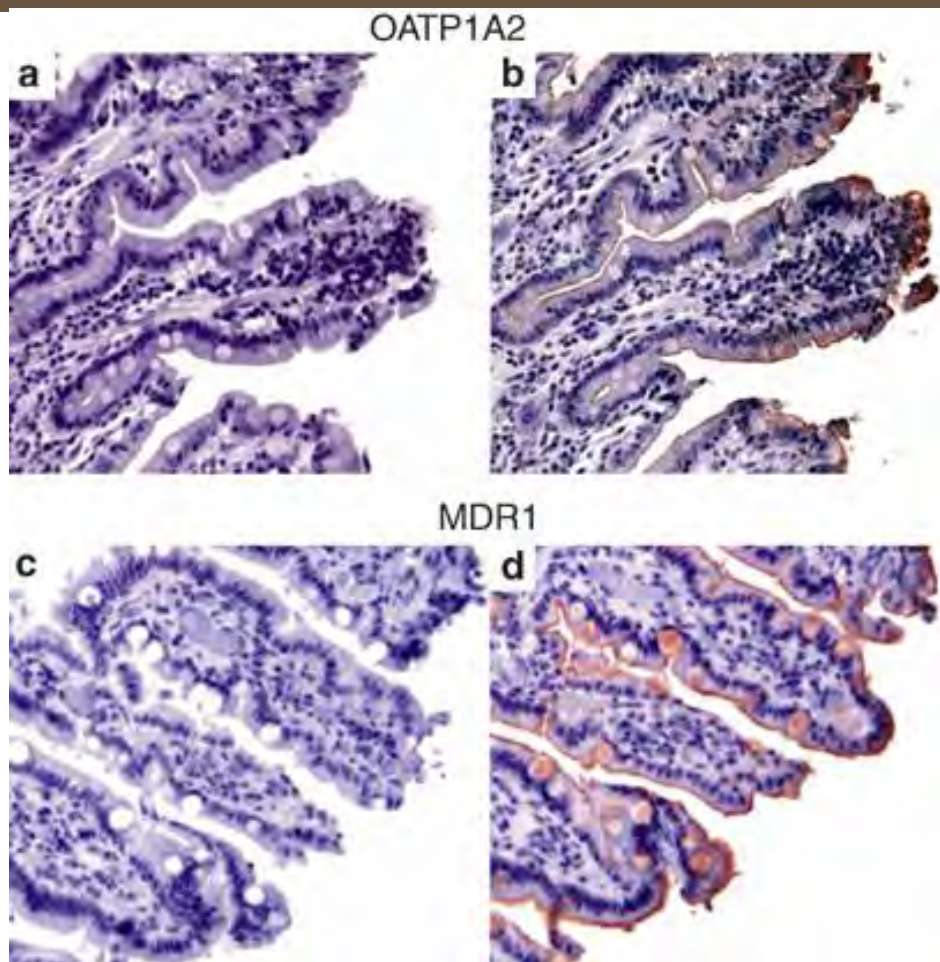
‡ ***Uptake intestinal transporters may facilitate the absorption of compounds with undesirable pharmaceutical properties, but are subject to inhibition and in some cases sequence polymorphisms***

# Inhibition of intestinal uptake transporters mimics the effects of drug metabolizing enzyme induction

	Effect on Victim drug	
Process	Plasma Levels	Clinical effect
Inhibition	Increased	Enhanced
<b>Induction</b>	<b>Decreased</b>	<b>Diminished</b>

- Inhibition of intestinal uptake transporters:
  - observed after single dosing
  - transient in nature
- Involvement of genetically polymorphic intestinal drug transporters may be an additional contributing factor to inter-individual variability in drug bioavailability

# Immunohistochemical staining of OATP1A2 and P-gp in human duodenal sections



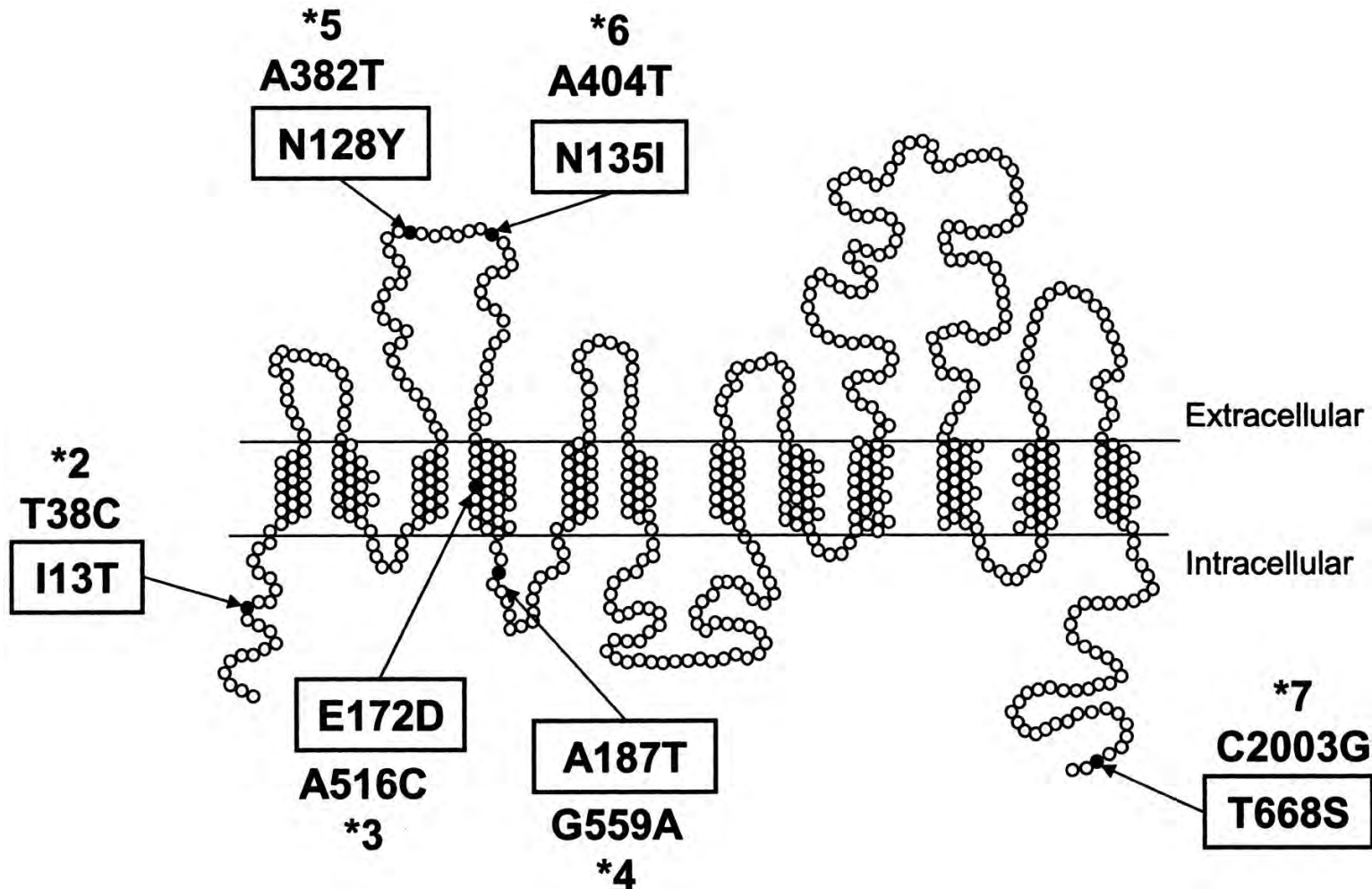
OATP1A2 expression is co-localized with P-gp on the brush border membrane of human enterocytes

Immunohistochemical staining of OATP1A2 and MDR1 in human duodenal sections

*Glaser H. et al. (2007) Clin Pharmacol Ther. 81(3); 362-370*

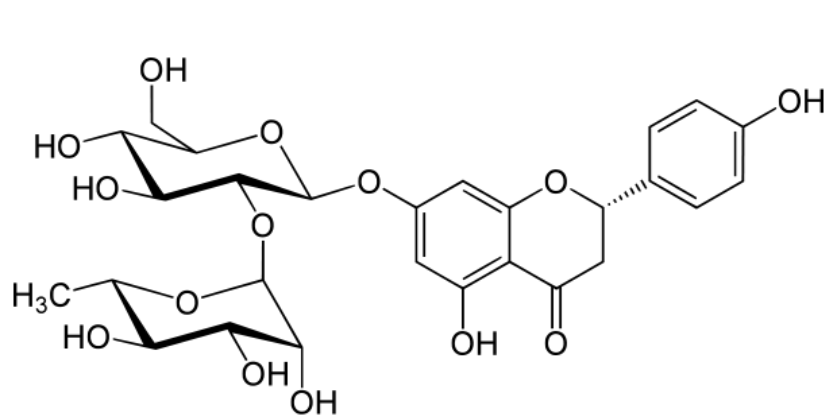
5 | NJDMDG Fall Symposium | Hanna | 06 October 2011 | Intestinal uptake transporters

# Location of non-synonymous amino acid substitutions along the predicted membrane topology of OATP1A2

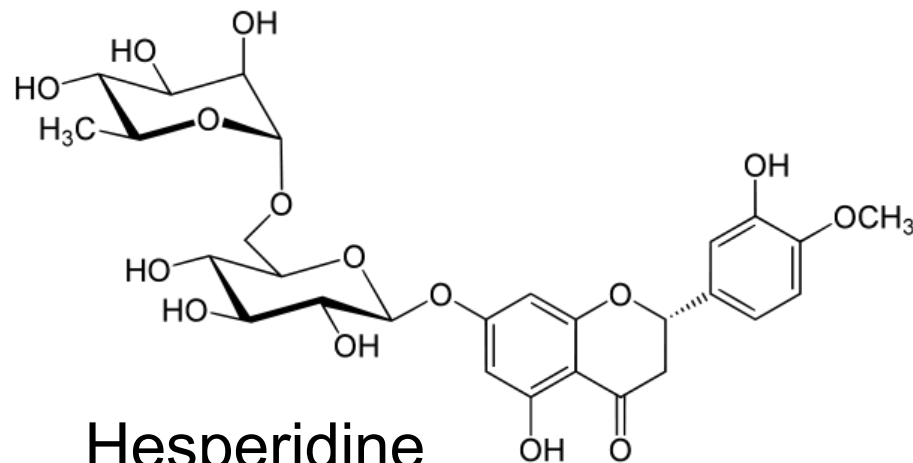


*Multiple polymorphisms identified in the OATP1A2 gene many of which result in reduced transport activity*  
Lee, W. et al. *J. Biol. Chem.* 2005;280:9610-9617

# Components of fruit juices that may inhibit transporter-mediated intestinal absorption



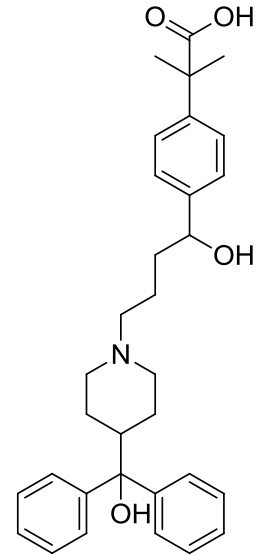
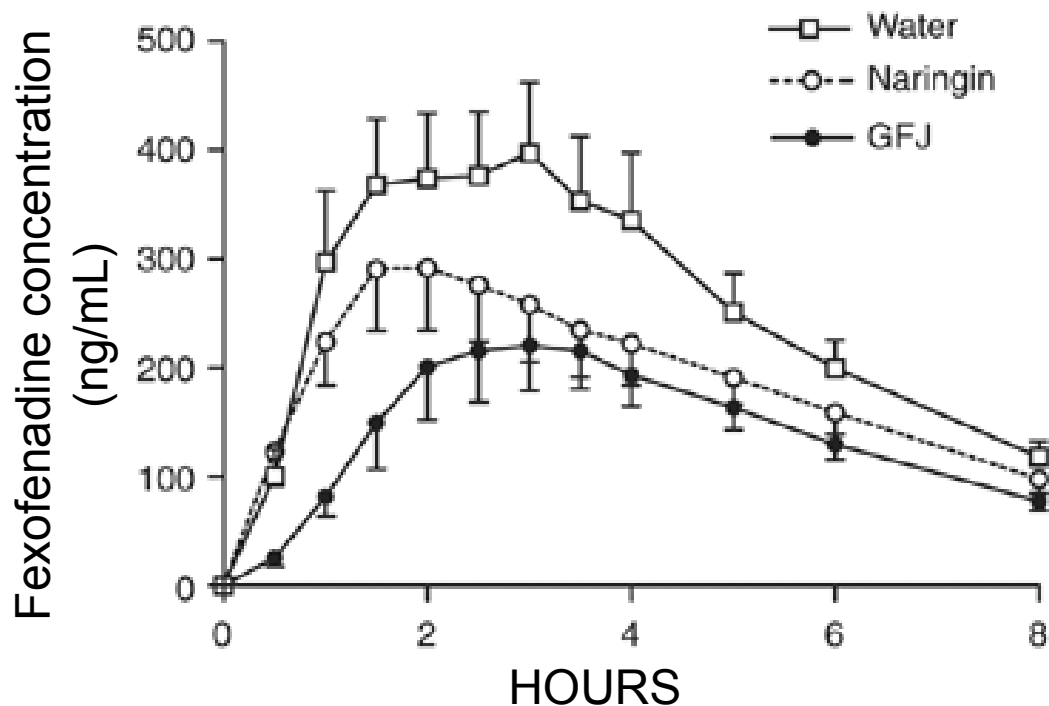
Naringin



Hesperidine

Flavonone glycosides in citrus juices are believed to be the ingredients responsible for altering the bioavailability of selected drugs (with more examples emerging) due to the inhibition of uptake processes in the intestine

# Effect of grape fruit juice or naringin on the oral bioavailability of fexofenadine



**Fexofenadine**

- Grapefruit juice reduced fexofenadine systemic exposure and  $C_{max}$
- The effect was transient in nature likely involving a competitive inhibition mechanism
- Naringin administration had an effect similar to that observed with Grapefruit juice.

Bailey DG. et al. (2007) *Clin Pharm Ther.* 81(4); 495-502

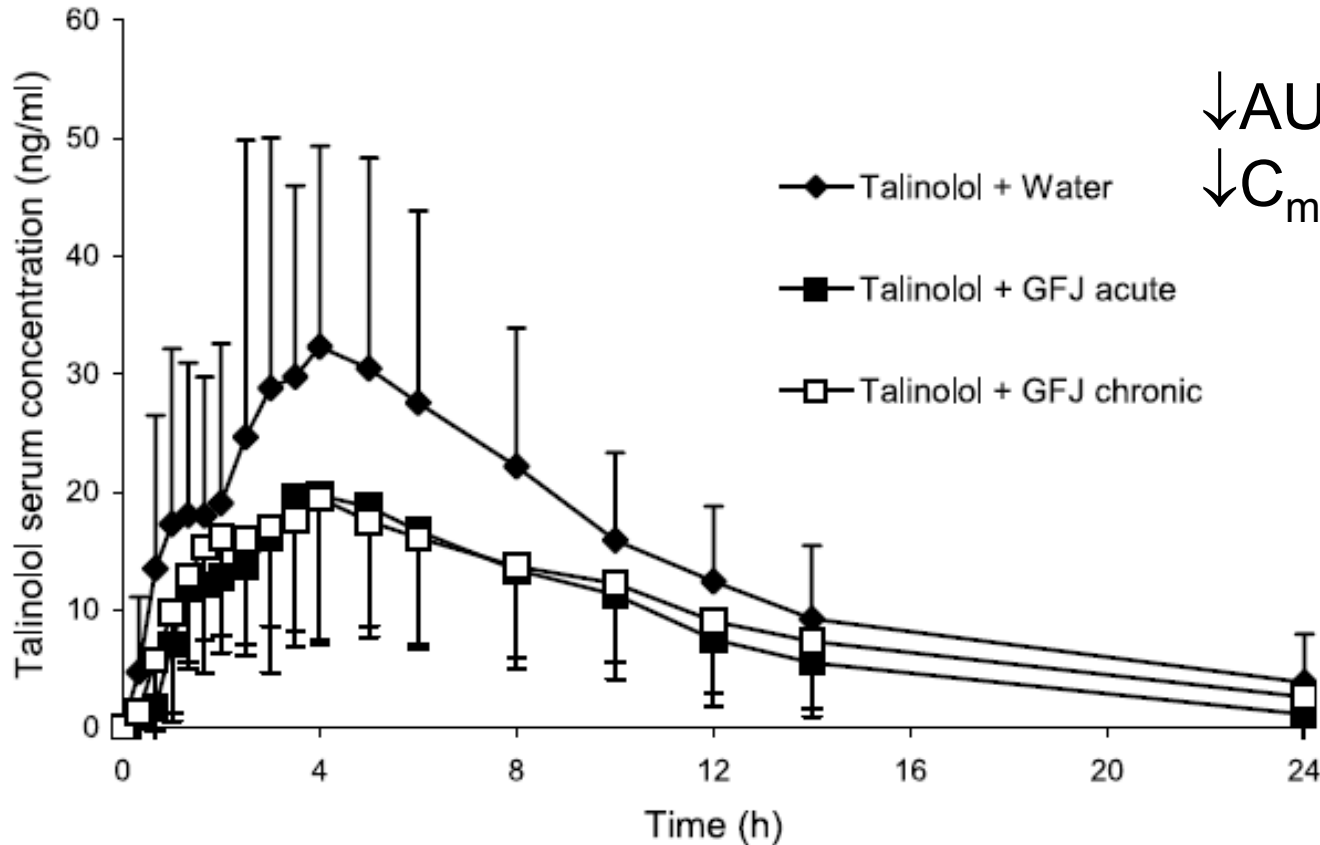


# Allegra® (fexofendine) package insert text regarding grapefruit juice effect on bioavailability

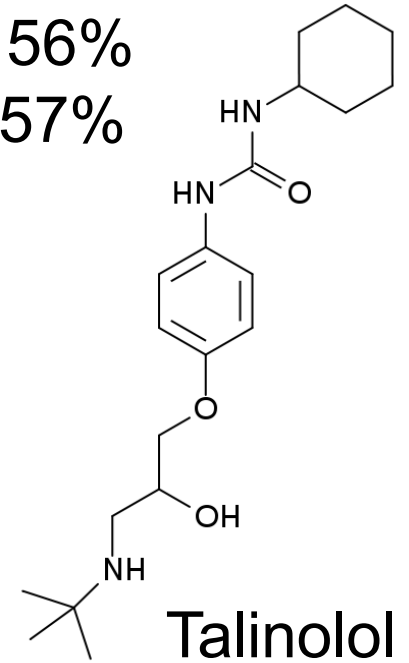
## ■ 7.3 Fruit Juices

- Fruit juices such as grapefruit, orange and apple may reduce the bioavailability and exposure of fexofenadine. This is based on the results from 3 clinical studies using histamine induced skin wheals and flares coupled with population pharmacokinetic analysis. The size of wheal and flare were significantly larger when fexofenadine hydrochloride was administered with either grapefruit or orange juices compared to water. Based on the literature reports, the same effects may be extrapolated to other fruit juices such as apple juice. The clinical significance of these observations is unknown. In addition, based on the population pharmacokinetics analysis of the combined data from grapefruit and orange juices studies with the data from a bioequivalence study, the bioavailability of fexofenadine was reduced by 36%. Therefore, to maximize the effects of fexofenadine, it is recommended that ALLEGRA tablets should be taken with water.

# Reduction in the plasma concentration of talinolol by grapefruit juice



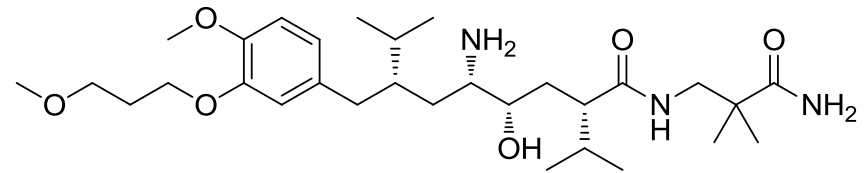
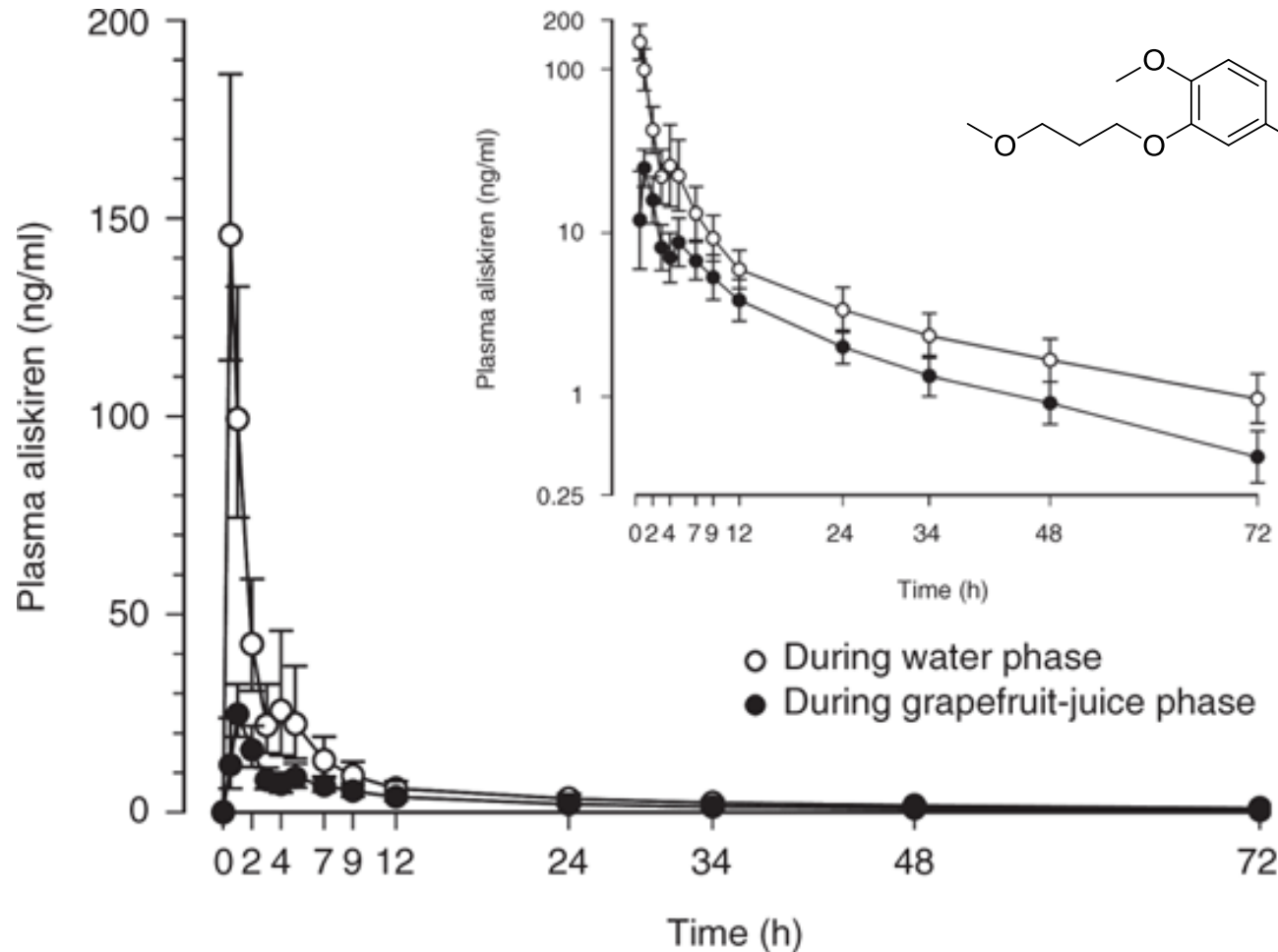
↓AUC: 56%  
↓C<sub>max</sub>: 57%



*Decreased systemic exposure to the non-metabolized drug, Talinolol, was likely due to a decrease in the intestinal uptake*

Schwarz et al., Clin Pharmacol Therap, 2005

# Reduction in the plasma concentration of aliskiren by grapefruit juice

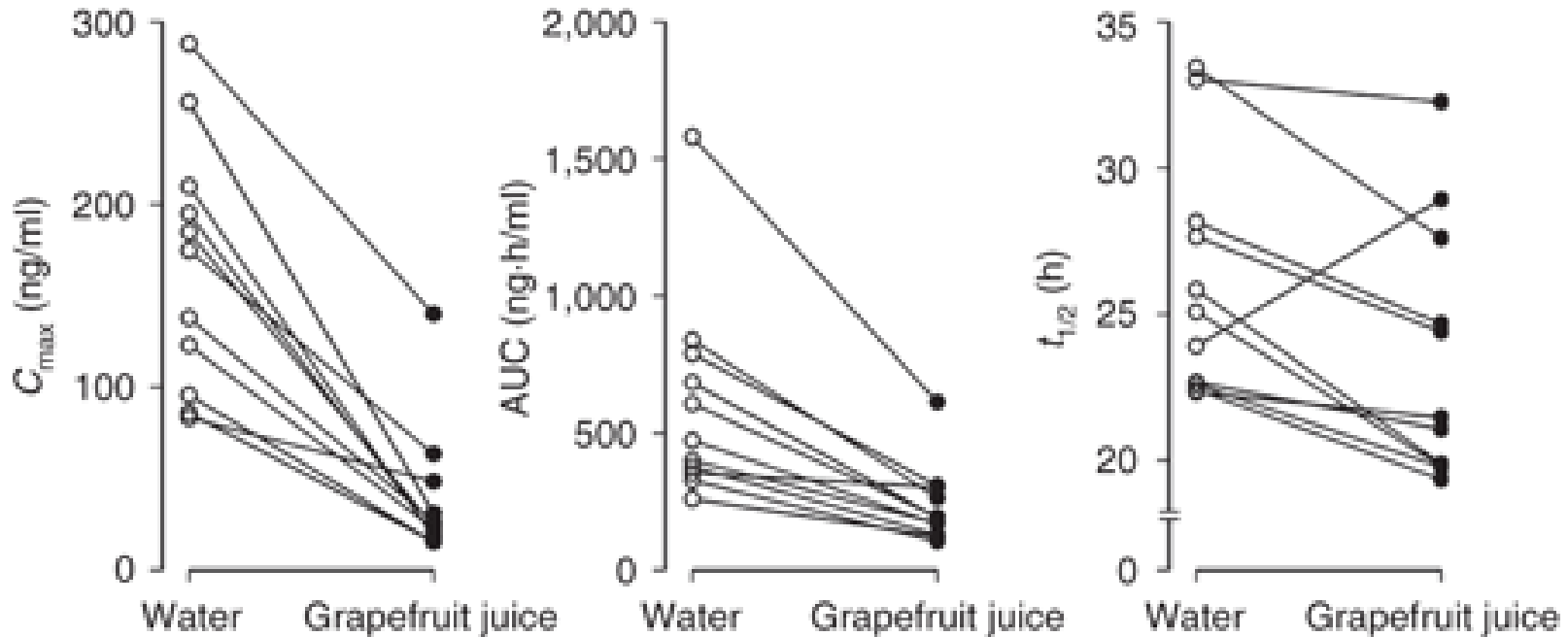


Aliskiren

↓AUC: 61%  
↓C<sub>max</sub>: 81%  
↓Urinary excretion: 66%

*Tapaninen et al., Clin. Pharm Therap. 2010*

# Individual $C_{max}$ , AUC and $t_{1/2}$ of Aliskiren in humans



*Tapaninen et al., Clin. Pharm Therap. 2010*

# Aliskiren Pharmacokinetics

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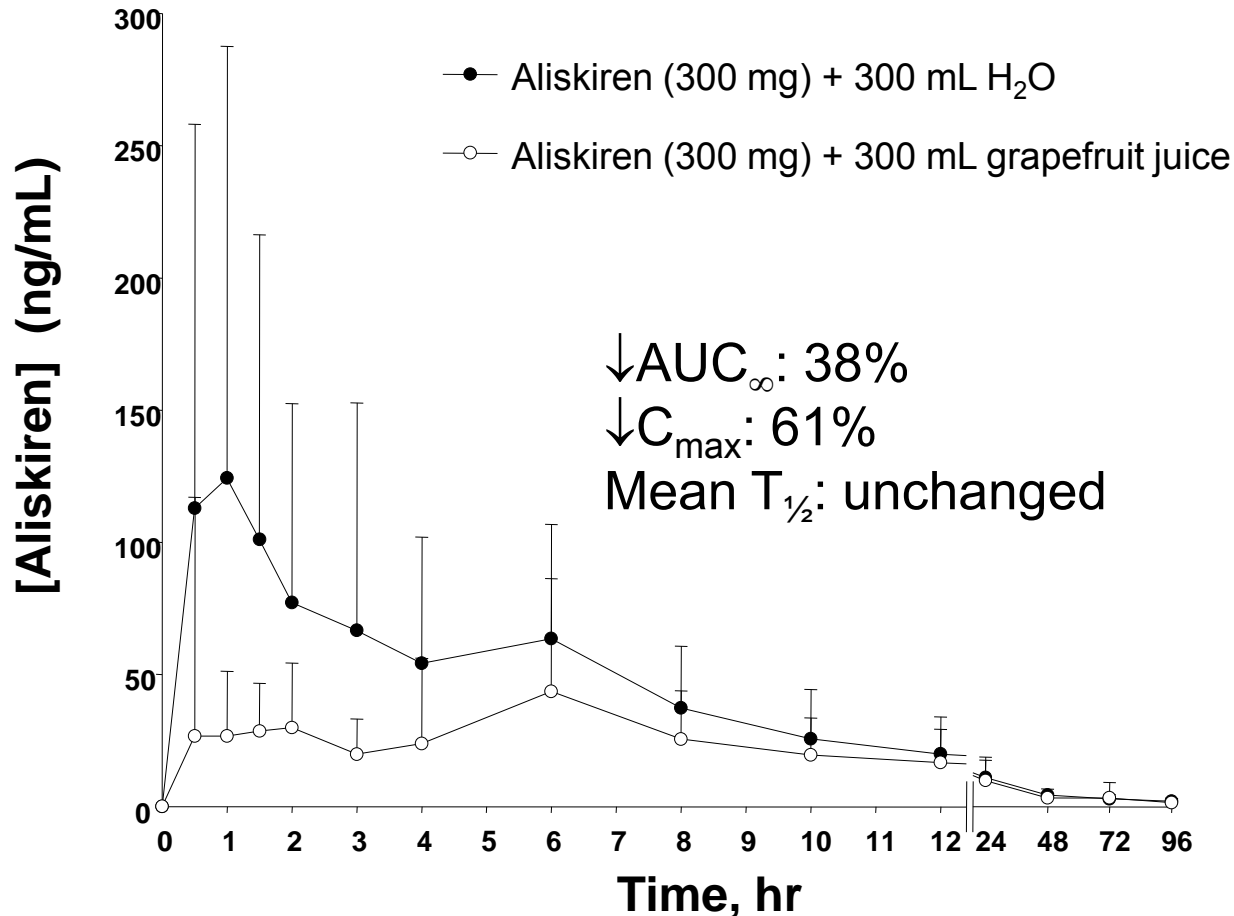
- Low permeability, P-gp substrate
- Low absorption in humans and animals
- Variable exposure in humans
- Decrease in exposure seen when given with grapefruit juice
- Decreased exposure maybe due to the inhibition of an uptake transporter in the intestine

# Clinical Study Design: Open-label, two-way crossover, single dose study in healthy subjects

Period 1 (Reference)		Period 2 (Test)
Aliskiren 300 mg single dose + 300 mL water	Washout (~10 days)	Aliskiren 300 mg single dose + Grapefruit Juice 300 mL

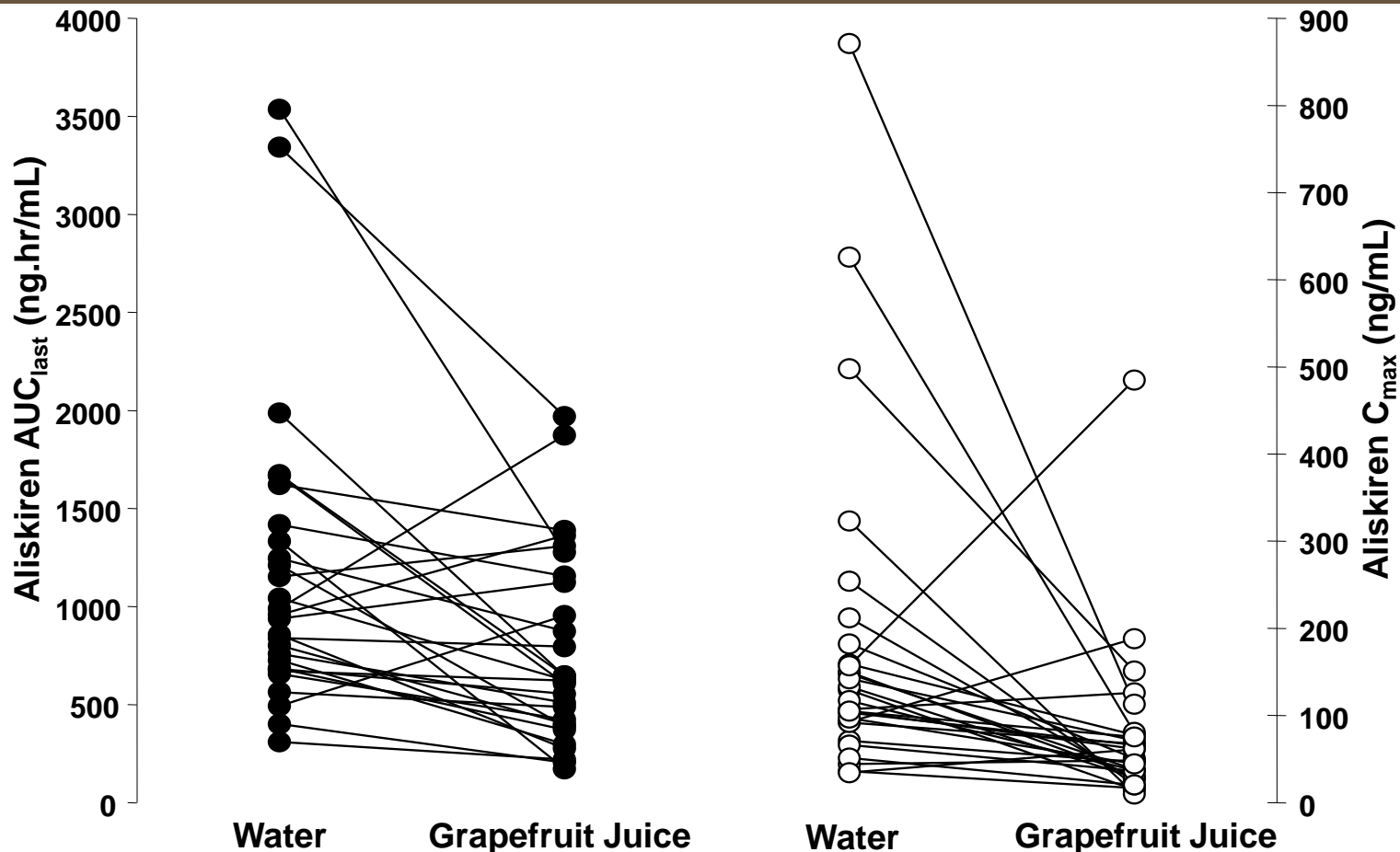
- 28 healthy male/female subjects
- Blood sampling on Period 1 and Period 2/Day 1: Pre-dose, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 24, 48, 72, 96 hours post-dose
- Analyte: Aliskiren
- PK variables: AUC<sub>inf</sub>, AUC<sub>last</sub>, C<sub>max</sub>, T<sub>max</sub> and T<sub>1/2</sub>
- Safety variables: Adverse Events, standard lab variables

# Effect of grapefruit juice on mean ( $\pm$ SD) Aliskiren plasma concentration time profile in humans



*The decrease in Aliskiren systemic exposure due to grapefruit juice was similar to that when given with food*

# Effect of grapefruit juice on the pharmacokinetic parameters of Aliskiren in humans



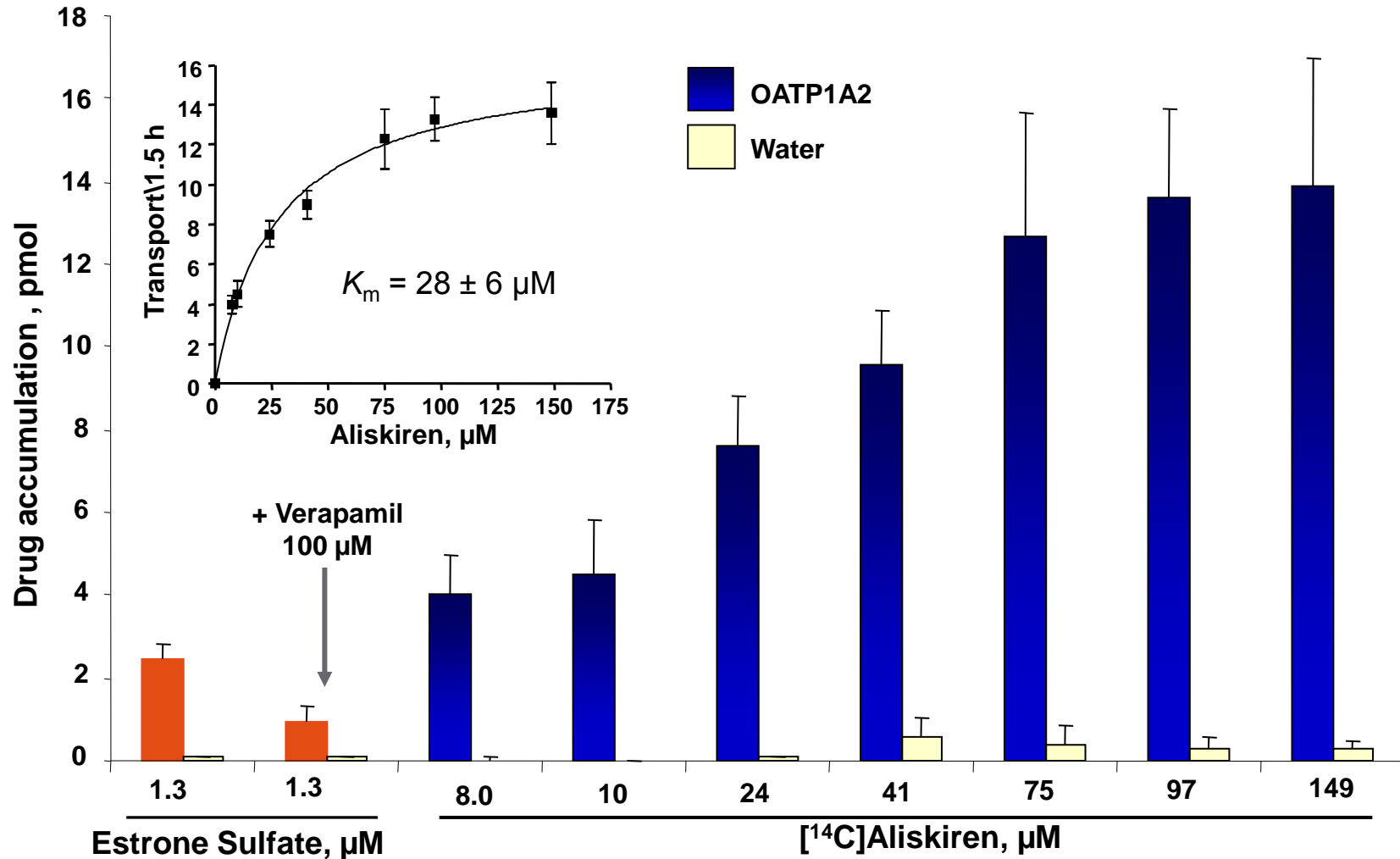
*Overall inter-subject variability associated with AUC and C<sub>max</sub> was similar between the test and reference treatments (n=28)*



# Effect of grapefruit juice on Aliskiren pharmacokinetics in human subjects

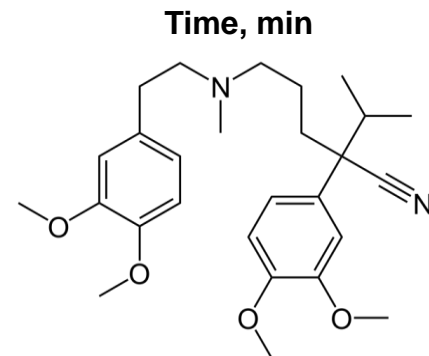
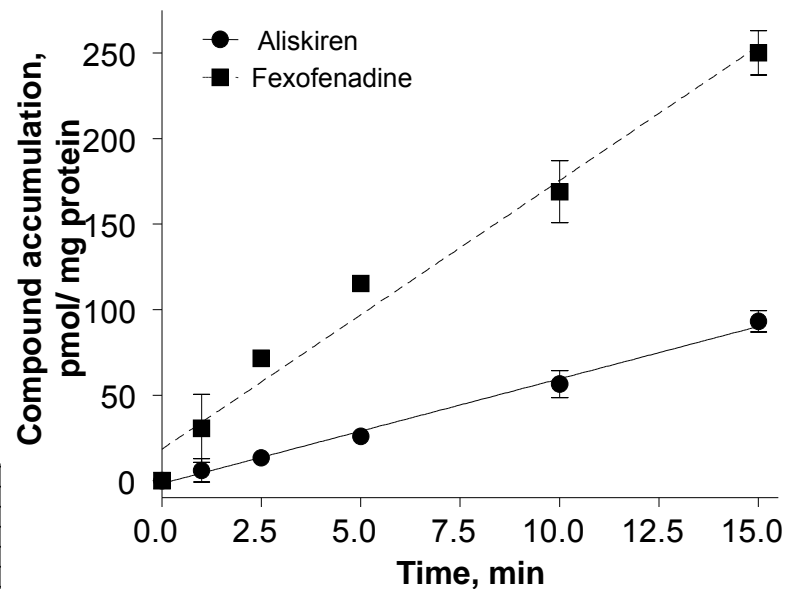
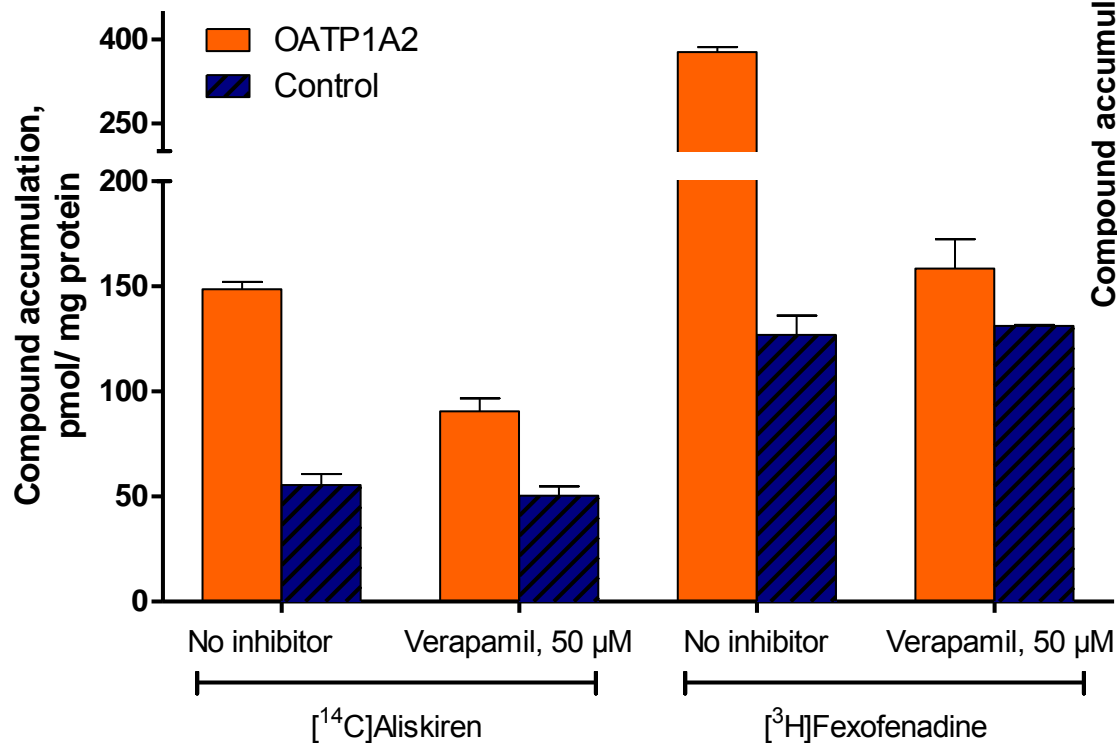
Treatment	Parameter	AUCinf (ng*hr/mL)	Cmax (ng/mL)	Tmax (hr)	T <sub>1/2</sub> (hr)
Reference	Mean ± SD	1236.5 ± 810	182.3 ± 189	1.64 ± 1.7	37.3
(Aliskiren, 300 mg + water)	Median, (%CV)	1008 (66)	123 (104)	1.00 (102)	35.5 (24)
Test	Mean ± SD	776.57 ± 493	74.72 ± 90.8	3.96 ± 3.3	31.7
(Aliskiren, 300 mg + Grapefruit juice)	Median, (%CV)	647.0 (64)	49.5 (122)	3.00 (84)	30.2 (28)

# Uptake of [<sup>14</sup>C]Aliskiren into *Xenopus* oocytes



***Aliskiren is an in vitro OATP1A2 substrate***

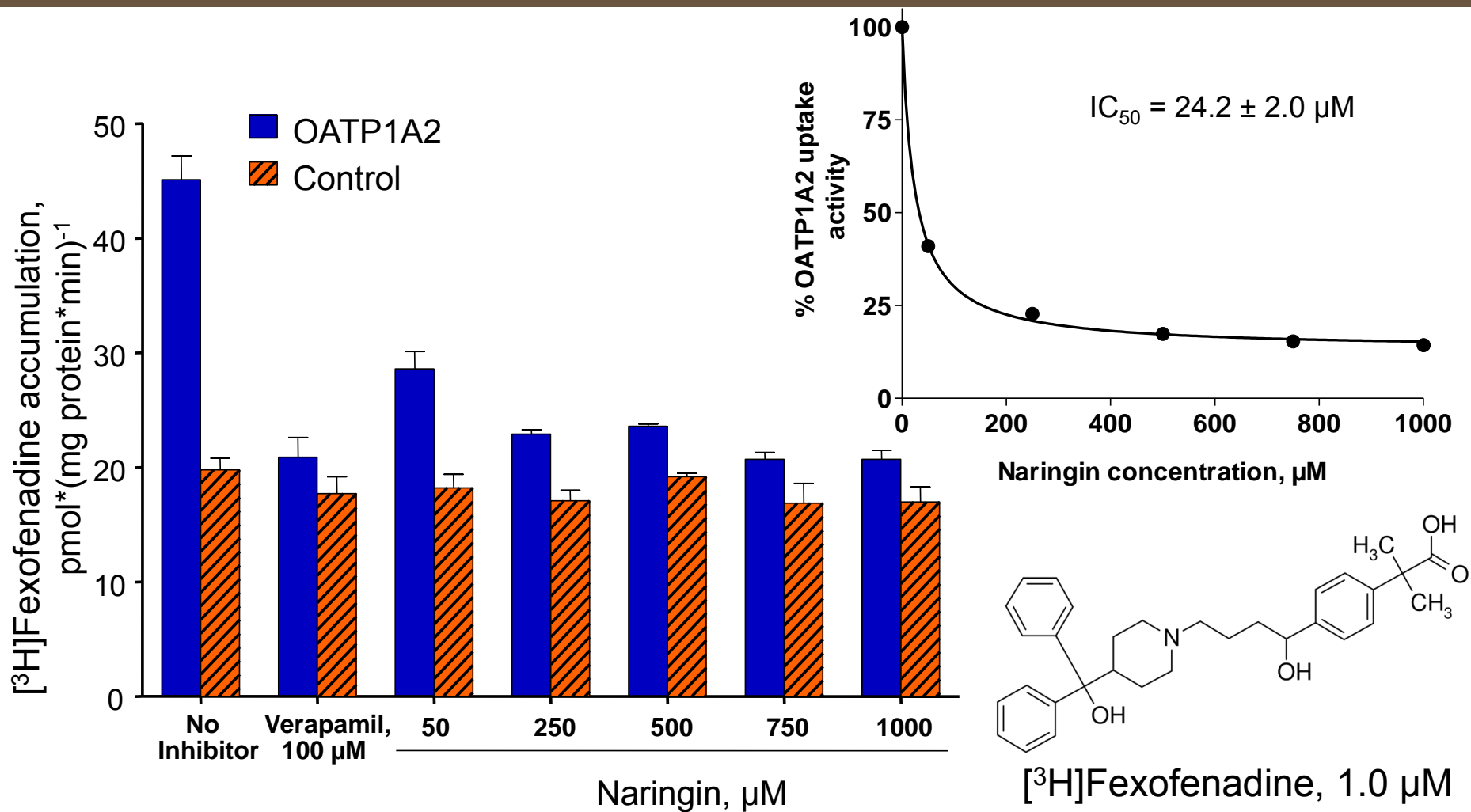
# [<sup>14</sup>C]Aliskiren and [<sup>3</sup>H]fexofenadine transport into HEK293 cells transiently expressing OATP1A2



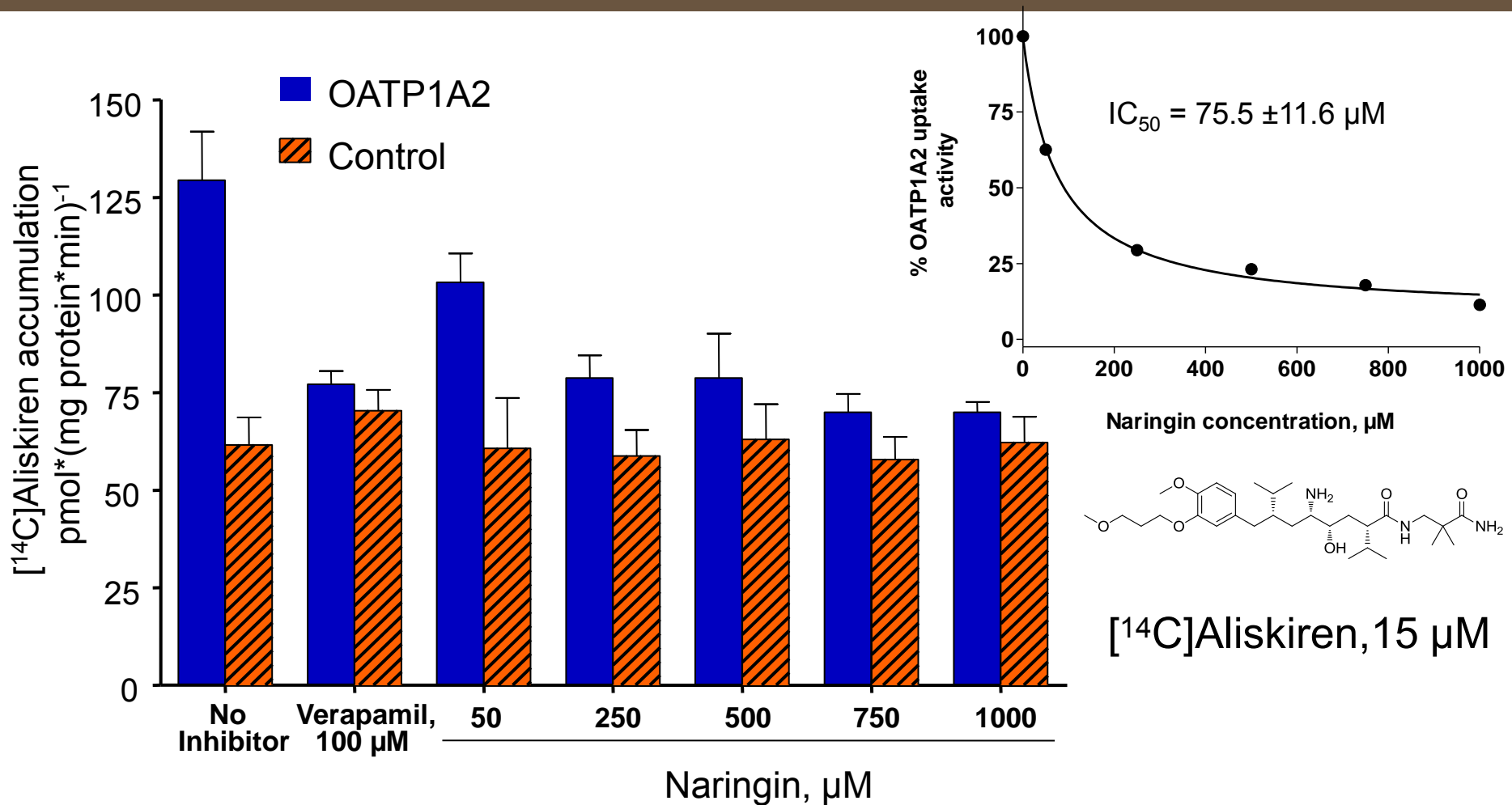
Verapamil  
(positive control inhibitor)

*Fexofenadine transport activity (10 μM) was ~2.5-fold higher than that of Aliskiren (10 μM)*

# Inhibition of [<sup>3</sup>H]fexofenadine uptake into control or OATP1A2 transfected cells by verapamil and naringin



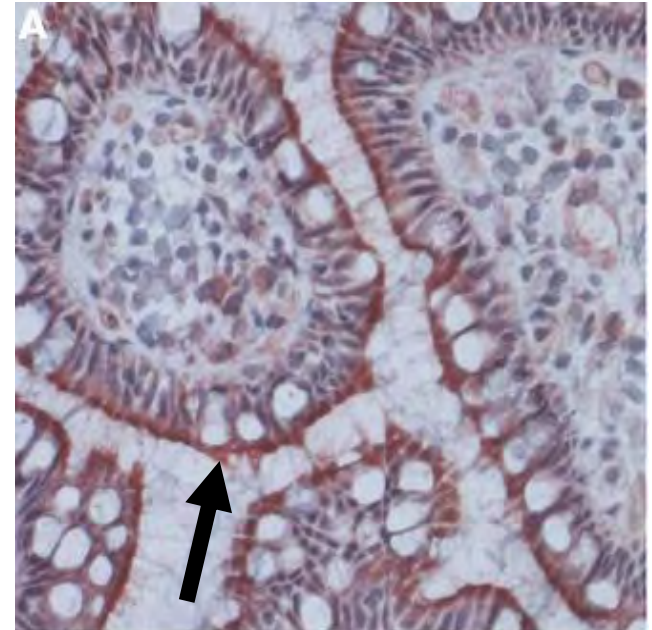
# Inhibition of [<sup>14</sup>C]Aliskiren uptake into control or OATP1A2 transfected cells by verapamil and naringin



*Reduced systemic exposure to Aliskiren when administered with grapefruit juice may be due to the inhibition of intestinal OATP1A2 uptake activity*

# Apical sodium-dependent bile acid transporter (ASBT; SLC10A2) is highly expressed in the ileum

- Substantial contribution to the enterohepatic circulation of bile acids
- Expressed in the ileum, cecum and kidney
- Mutations in ASBT gene result in bile acid mal-absorption and congenital diarrhea
- Inhibition of ASBT results in changes in cholesterol and bile acid homeostasis

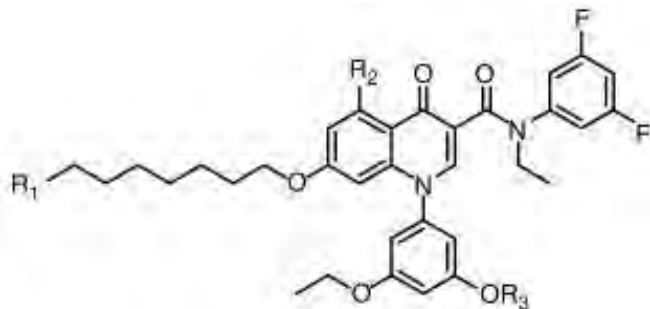


**Immuno-reactive  
ASBT in human ileal slices**

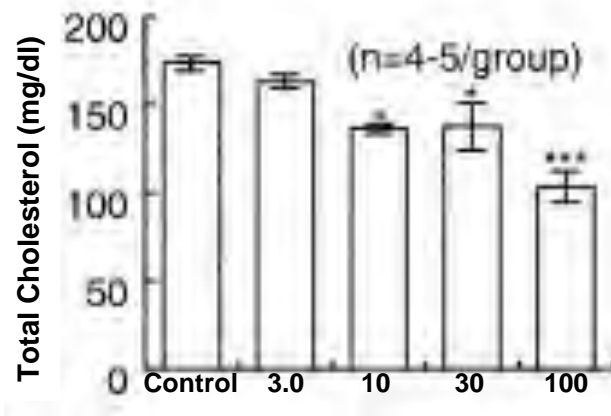
*Adapted from: Hruz et al., Gut 2006; 55:395-402*

# ASBT is a promising target for non-systemically acting hypolipidemic drugs.

- Bile acids regulate the activity of hepatic cholesterol-7- $\alpha$ -hydroxylase: *the rate-limiting step in the conversion of cholesterol to bile acids*
- ASBT inhibitors provide a new therapeutic approach for the treatment of hypercholesterolemia.
- ASBT inhibitors are generally not absorbed resulting in low incidence of systemic side effects.
- Inhibition of ASBT activity by R-146224 (Sankyo) reduced total plasma cholesterol levels in hamsters and monkeys



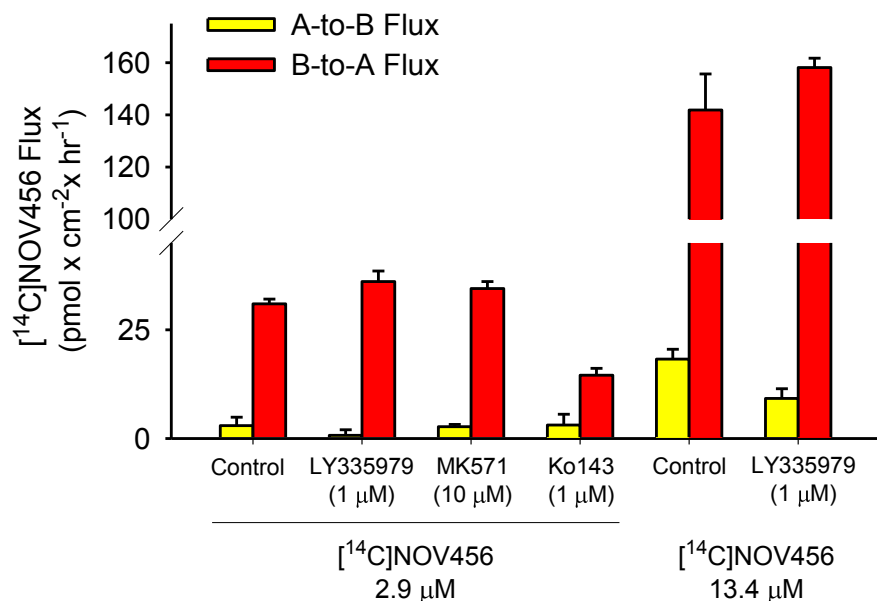
R-146224 (Sankyo)



R-146224 (mg/kg/day)

Adapted from Kitayama et al., *Eur J Pharm* (2006) 539:89

# NOV456 *in vitro* transport properties and interaction with drug efflux transporters



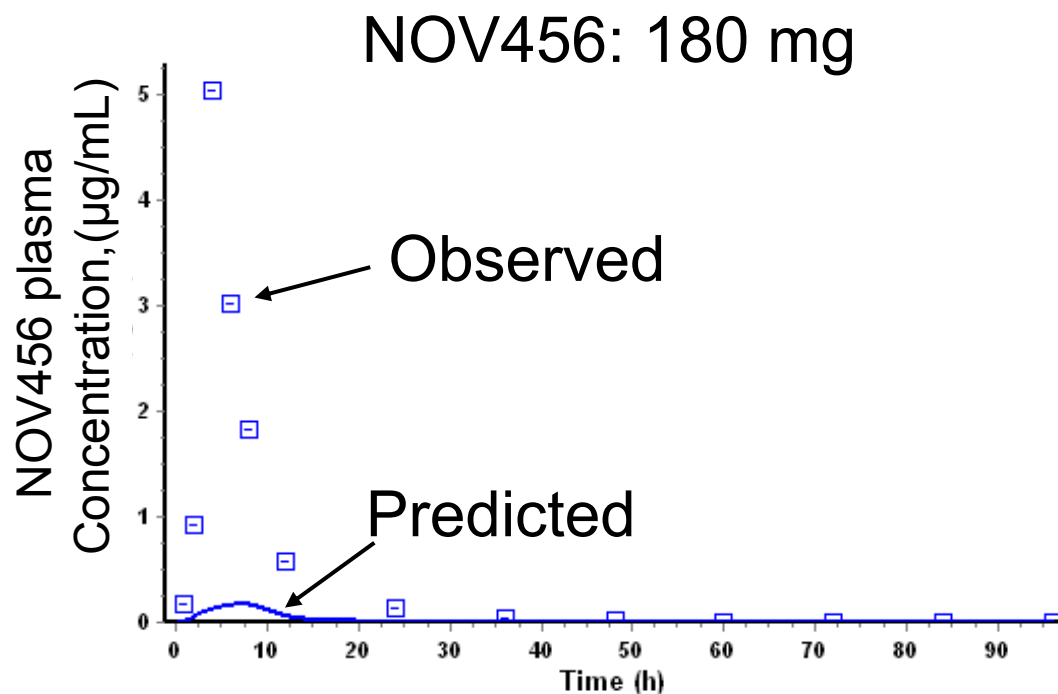
Compound, Concentration	Caco-2 Permeability *10 <sup>-5</sup> (cm*min <sup>-1</sup> )		Efflux ratio
	Ap→Bl	Bl→Ap	
NOV456, 2.9 μM	1.7 ± 1.1	17.9 ± 0.6	10.5
NOV456, 13.4 μM	2.3 ± 0.3	17.6 ± 1.7	7.65
Mannitol, 3.6 μM	7.8 ± 1.0	4.8 ± 0.4	0.61
Propranolol, 5.2 μM	94 ± 18	106 ± 13	1.1

## Conclusions:

- NOV456 is a low permeability compound and appears to be actively transported ( $P_{app}$  ratio > 2) across Caco-2 monolayers
- NOV456 pharmacokinetics may be influenced by transporters

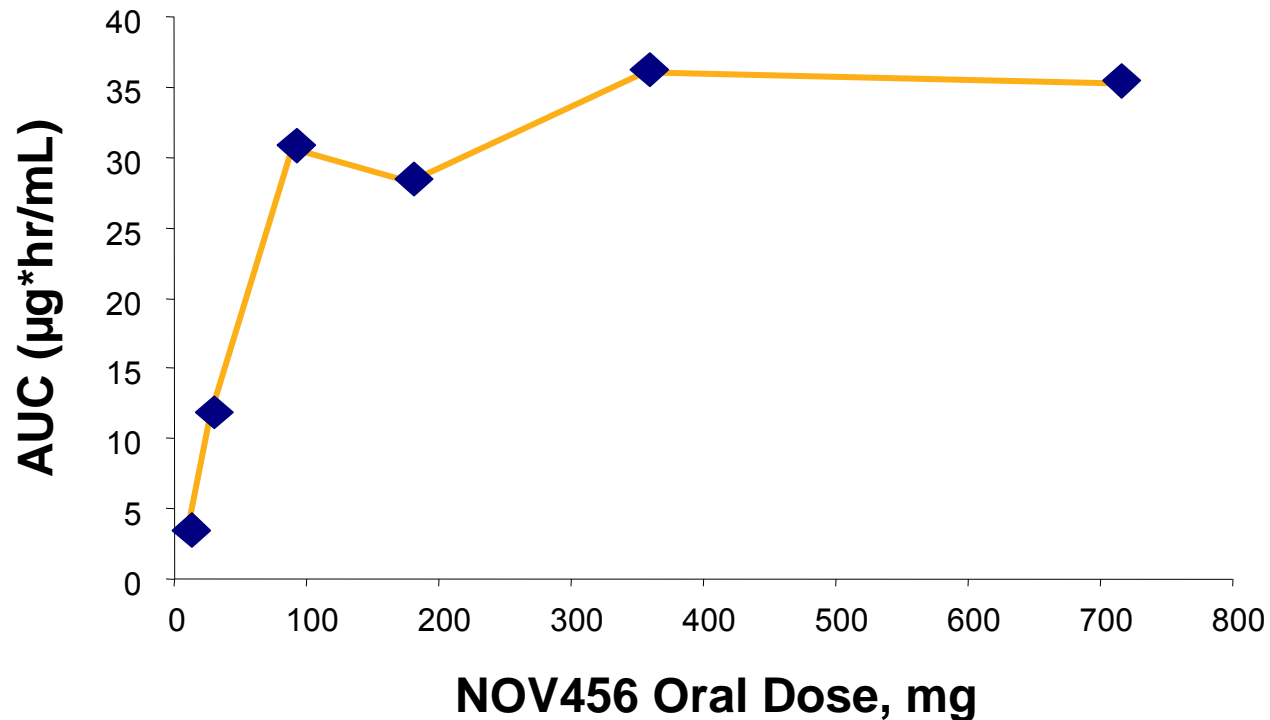


# Pharmacokinetic simulations without incorporating absorptive transport mechanisms underestimates the observed human exposure of NOV456



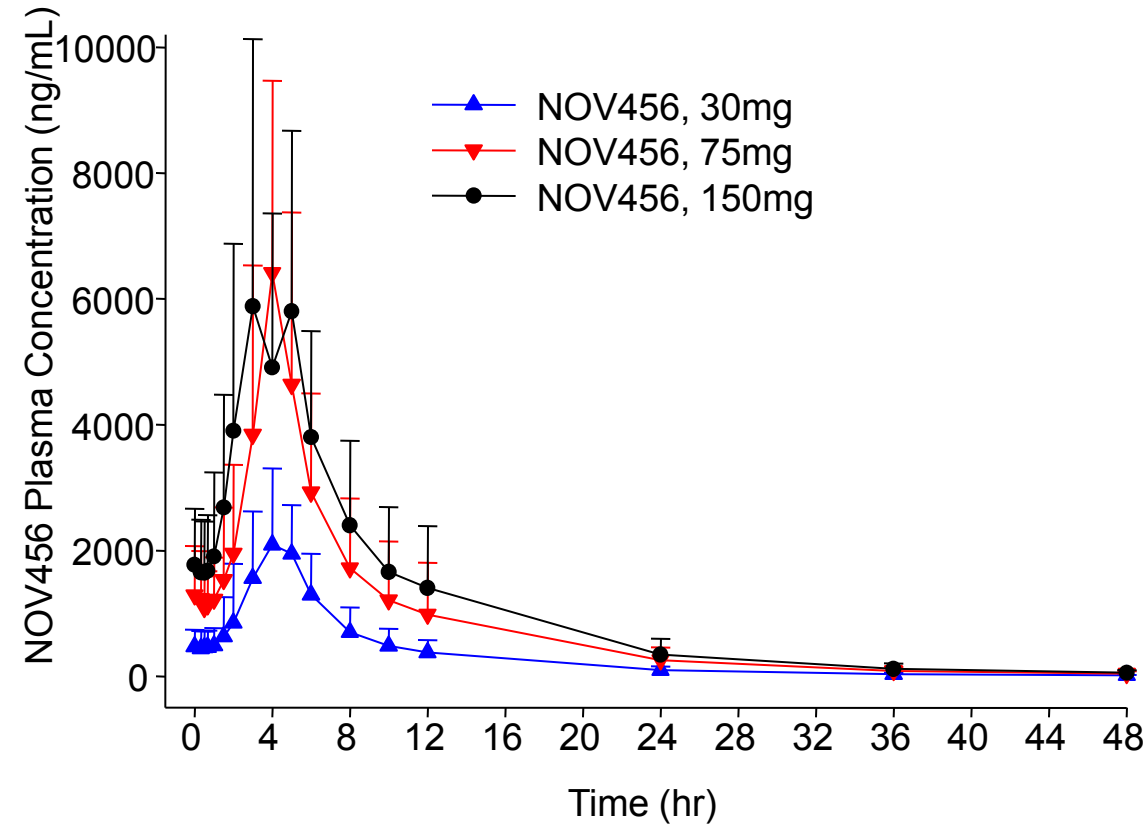
*Gastroplus<sup>TM</sup> simulations of NOV456 plasma-concentration time profiles using solubility and passive permeability inputs, significantly under-predicted the actual profiles observed in humans*

# NOV456 displays non-linear Pharmacokinetics following oral administration to humans (10-700 mg)

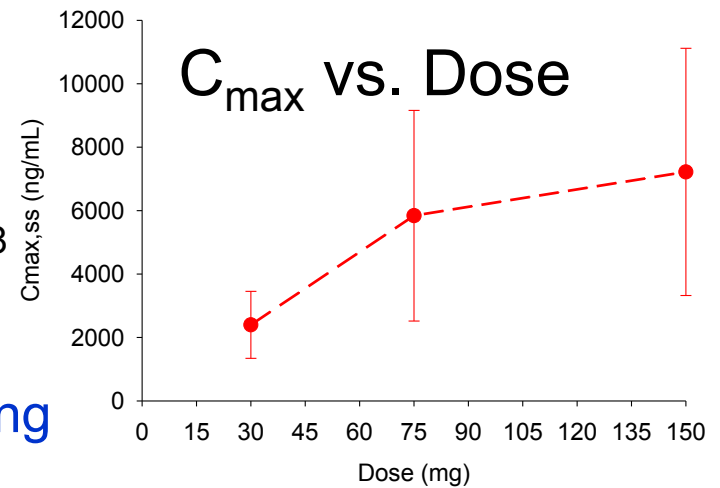
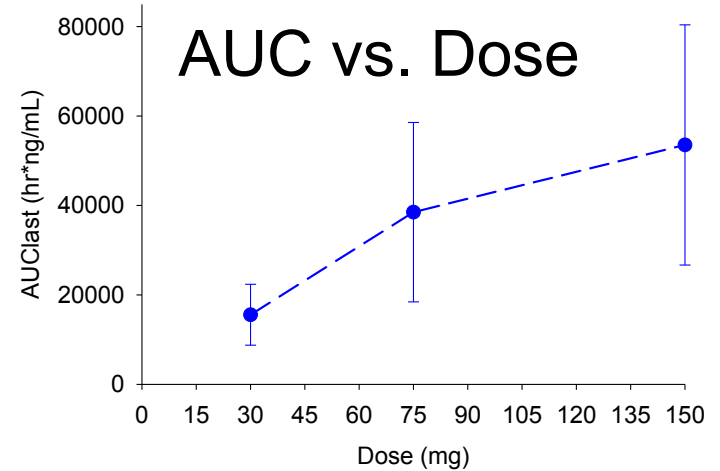


- Dose under-proportionality is observed at doses greater than 90 mg in humans

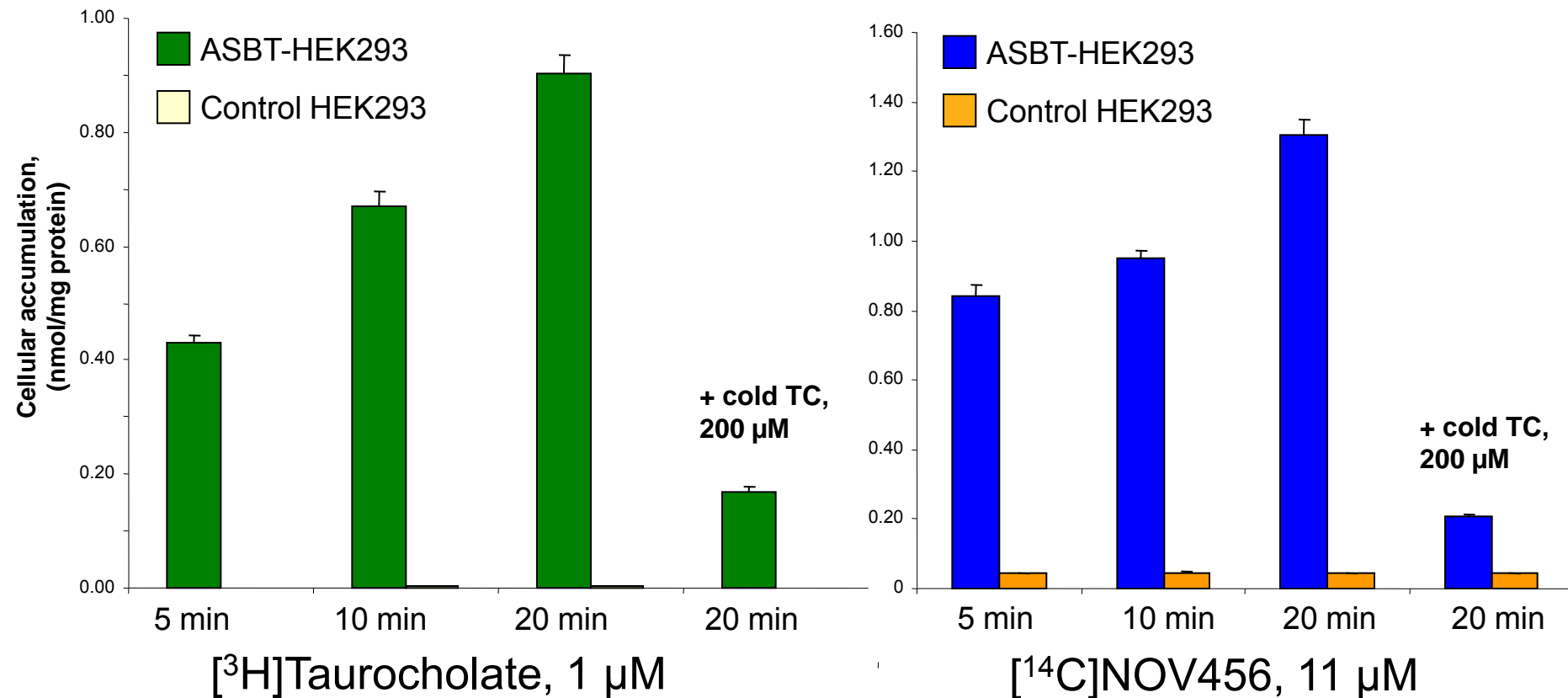
# NOV456 dose under-proportionality



In all cases  $T_{max}$  is delayed (2 -6 h), suggesting absorption in lower segments of the GI tract

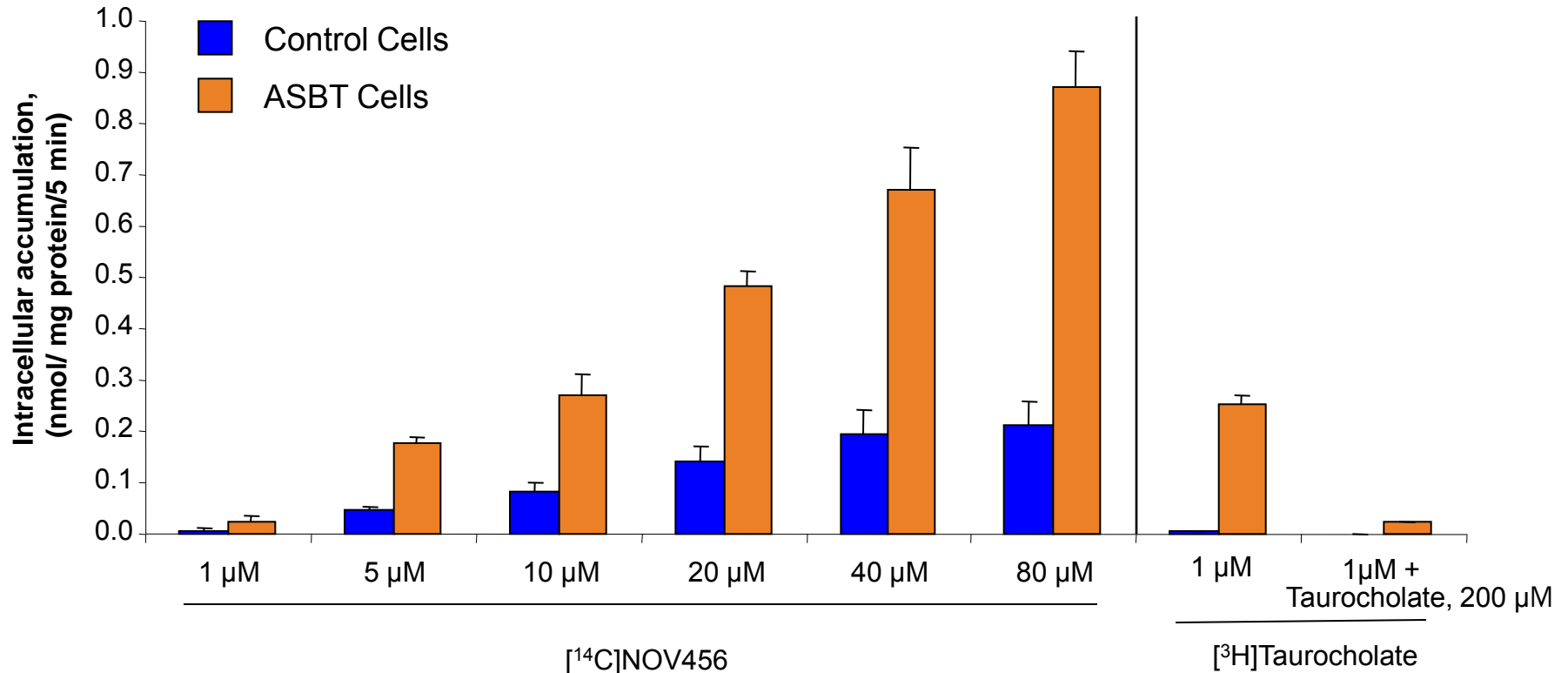


# Time dependent [<sup>3</sup>H]taurocholate (TC) or [<sup>14</sup>C]NOV456 accumulation in control or ASBT-transfected HEK293 cells



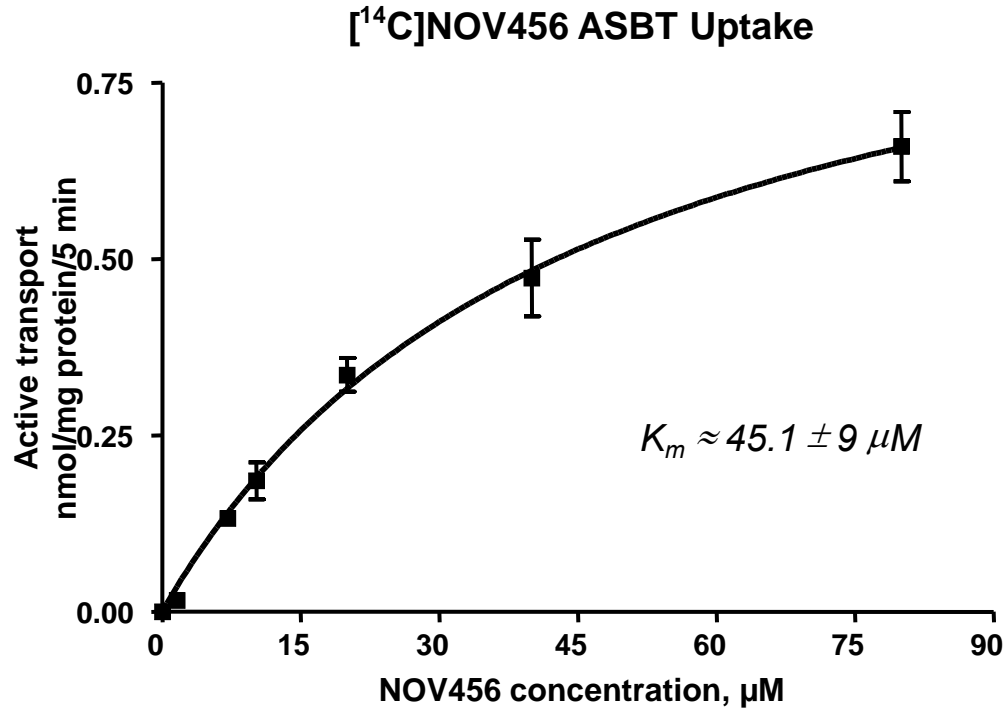
- [<sup>14</sup>C]NOV456 or [<sup>3</sup>H]Taurocholate uptake was “near” linear up to 20 min into ASBT-transfected cells
- Marked reduction in radioactivity accumulation was seen in the presence of non-radiolabeled taurocholate (competitive inhibition)
- ASBT may play a role in the intestinal absorption of NOV456

# [<sup>14</sup>C]NOV456 (1-80 μM) uptake into control or ASBT transfected HEK293 cells (5 min incubations)



- [<sup>14</sup>C]NOV456 uptake was higher in ASBT-transfected cells compared to control cells
- NOV456 was soluble within the concentration range tested
- An active uptake mechanism can be seen in control cells as well as the ASBT-transfected cells

# NOV456- ASBT uptake kinetics



- [<sup>14</sup>C]NOV456 transport (Accumulation in ASBT cells-accumulation in control cells) was incompletely saturated up to 80  $\mu\text{M}$ .
- The  $K_m$  associated with the active transport was approximately 45  $\mu\text{M}$ .
- In comparison, a 100 mg NOV456 dose dissolved in 250 mL  $\text{H}_2\text{O}$  yields a concentration  $\sim$  760  $\mu\text{M}$

# Prediction of NOV456 pharmacokinetics (*oral doses 178-712 mg*) following the incorporation of ASBT uptake kinetics

Compound: NOV456, potassium salt

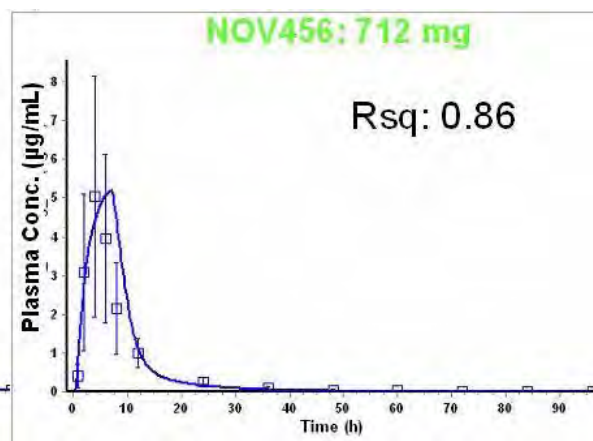
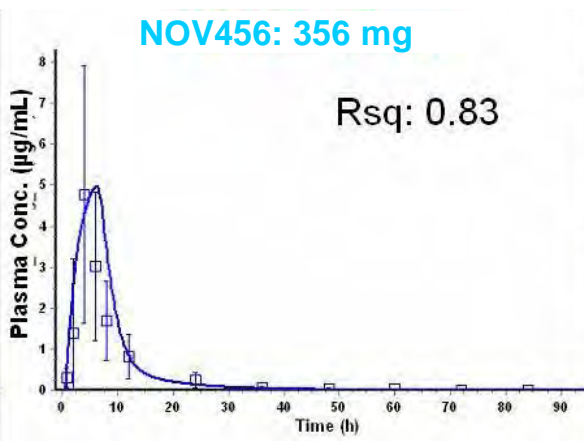
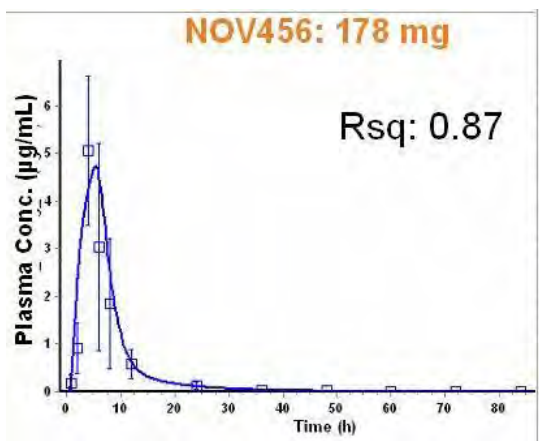
Solubility: 16 ng/mL at pH 1.0

ASBT parameters:  $V_{max}$  0.377 (optimized),  $K_m$  23.5 (converted from *in vitro* data)

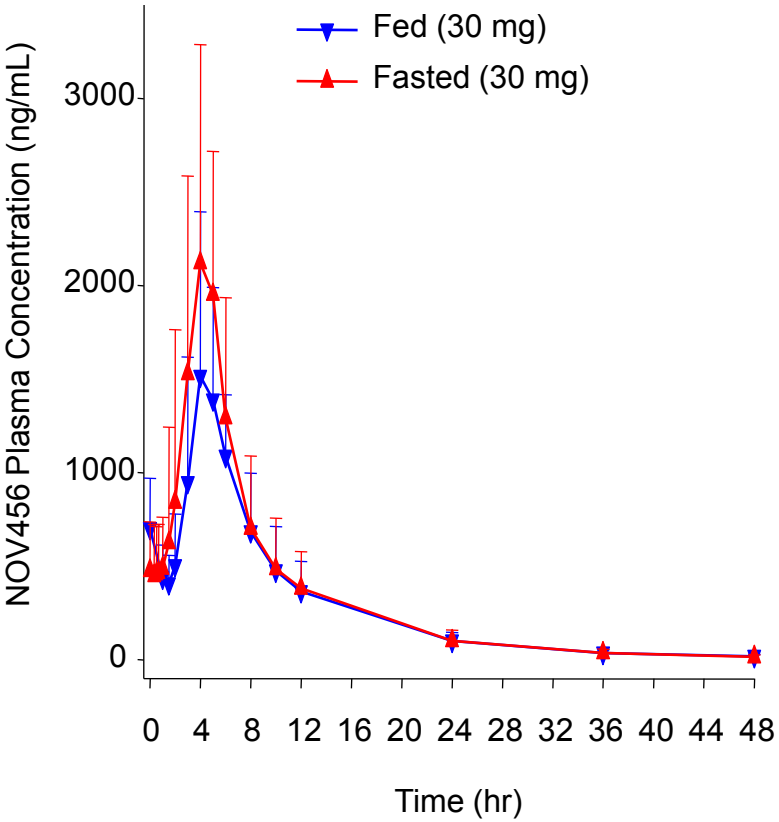
PK model: two compartment

GIT physiology: optimized logD model, fasted

	Observed	Predicted		Observed	Predicted		Observed	Predicted
Dose (mg)	178			356			712	
$T_{max}$ (h)	4-6	5.4		4-6	6.3		4-6	7
$C_{max}$ ( $\mu\text{g/mL}$ )	5.04	4.7		4.77	5.0		5.1	5.2
AUC ( $\text{ng}\cdot\text{h/mL}$ )	29800	33800		34300	39500		41200	45300
Fa(%)		<b>37.8</b>			<b>22</b>			<b>12.7</b>
F (%)	<b>31.6</b>	<b>35.9</b>		<b>16.8</b>	<b>21</b>		<b>9.2</b>	<b>12</b>



# Negative food effect on NOV456 after a light meal



Parameter	Fasted	Fed.
Dose (mg)	30	30
T <sub>max</sub> (h)	4-6	4-7
C <sub>max</sub> (µg/mL)	2.4 ± 1.0	1.7 ± 0.74
AUC (µg*h/mL)	15.6 ± 6.7	13.4 ± 4.9

- Reduced exposure and C<sub>max</sub> in the presence of food
- Negative food effects may be due to competitive inhibition of ASBT by bile acids



# Conclusions/Summary

- Nutrient and uptake transporters in the gastrointestinal tract (e.g. OATP1A2 and ASBT) may participate in the transport of xenobiotics (e.g. fexofenadine, aliskiren, and NOV456).
- Inhibition of such processes can represent a mechanism underlying drug-drug, or drug-diet interactions.
- Possible hallmarks of uptake transporters involvement include:
  - Poor *in silico* to *in vivo* prediction of the extent of absorption
  - Lack of dose proportionality (dose under-proportionality)
  - Marked Inter-individual difference in pharmacokinetics
  - Negative food effects
- A better understanding of uptake transport activity is needed to evaluate the role played by such processes in influencing the pharmacokinetics of new drug candidates

# Acknowledgments

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- Arpine Vapurucyan
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- Ryan Pelis
- Kenneth Kulmatycki
- Wen Lin