Quantitative Evaluation of the Effect of P-Glycoprotein on Oral Drug Absorption

-Assessment of Drug Permeability to Rat Small Intestine-

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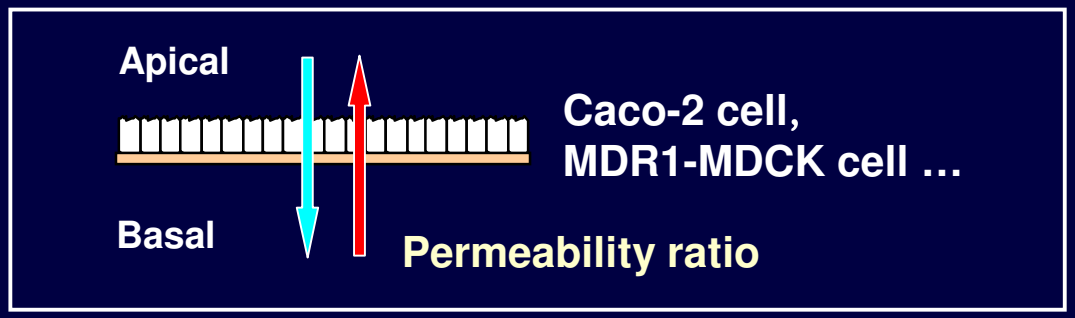
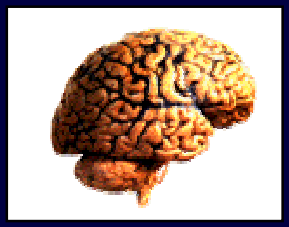
PURPOSE

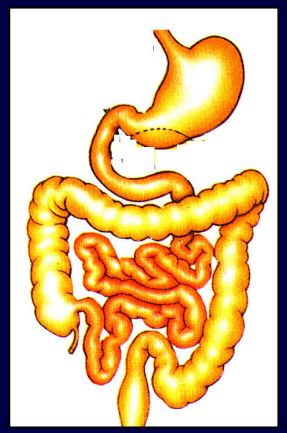
Transport Experiments by using P-gp Expressing Cell Monolayers

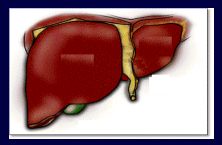




Transport Rate = f (K_m, V_{max}, $P_{passive}$)

- Drug Concentration
- P-gp Expression Level
- Affinity to P-gp
- Passive Membrane Permeability
- Cellular Accumulation

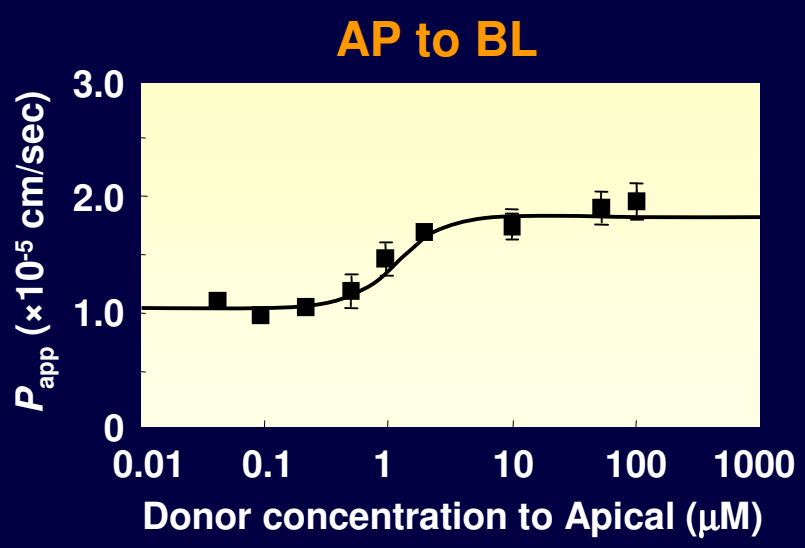


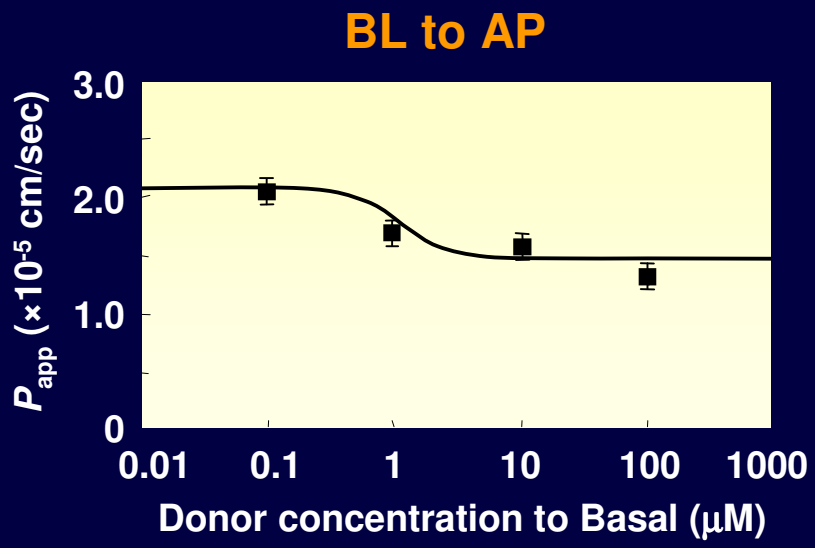


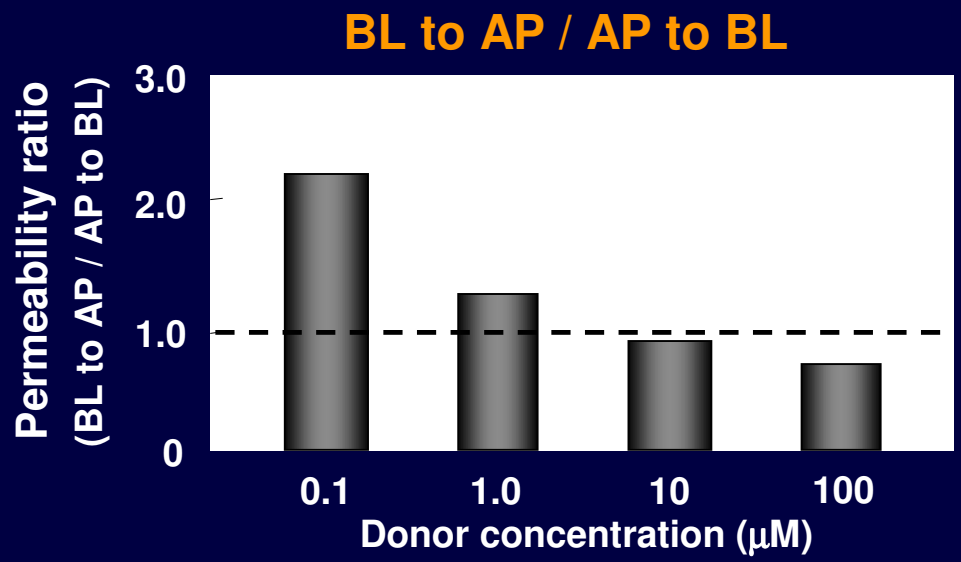
＜Impact of P-gp on *in vivo* Oral Drug Absorption＞

Development of kinetic model that can predict the *in vivo* absorption of P-glycoprotein substrate drugs from *in vitro* data.

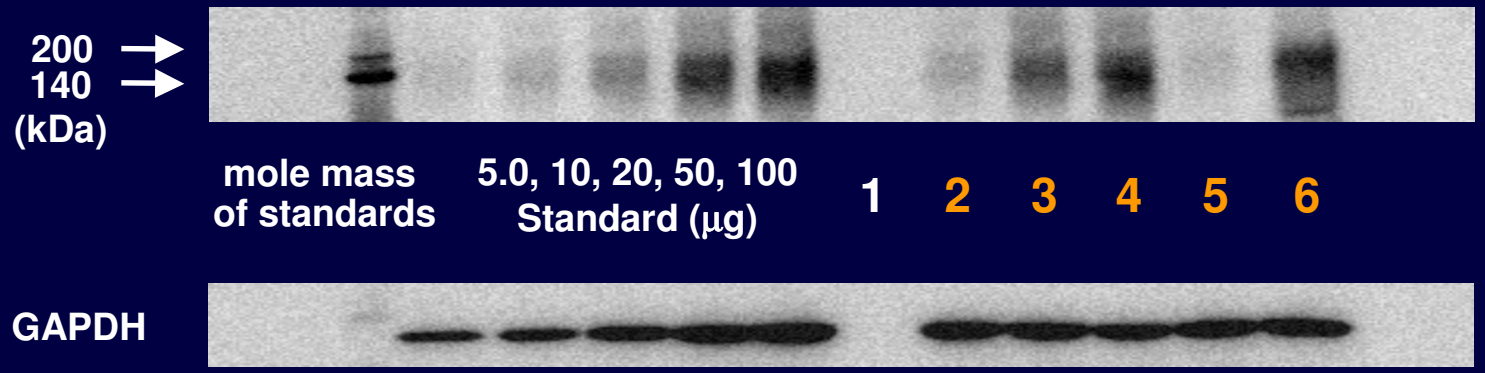
Effect of donor concentration on AP to BL and BL to AP transport of quinidine in Caco-2 monolayers







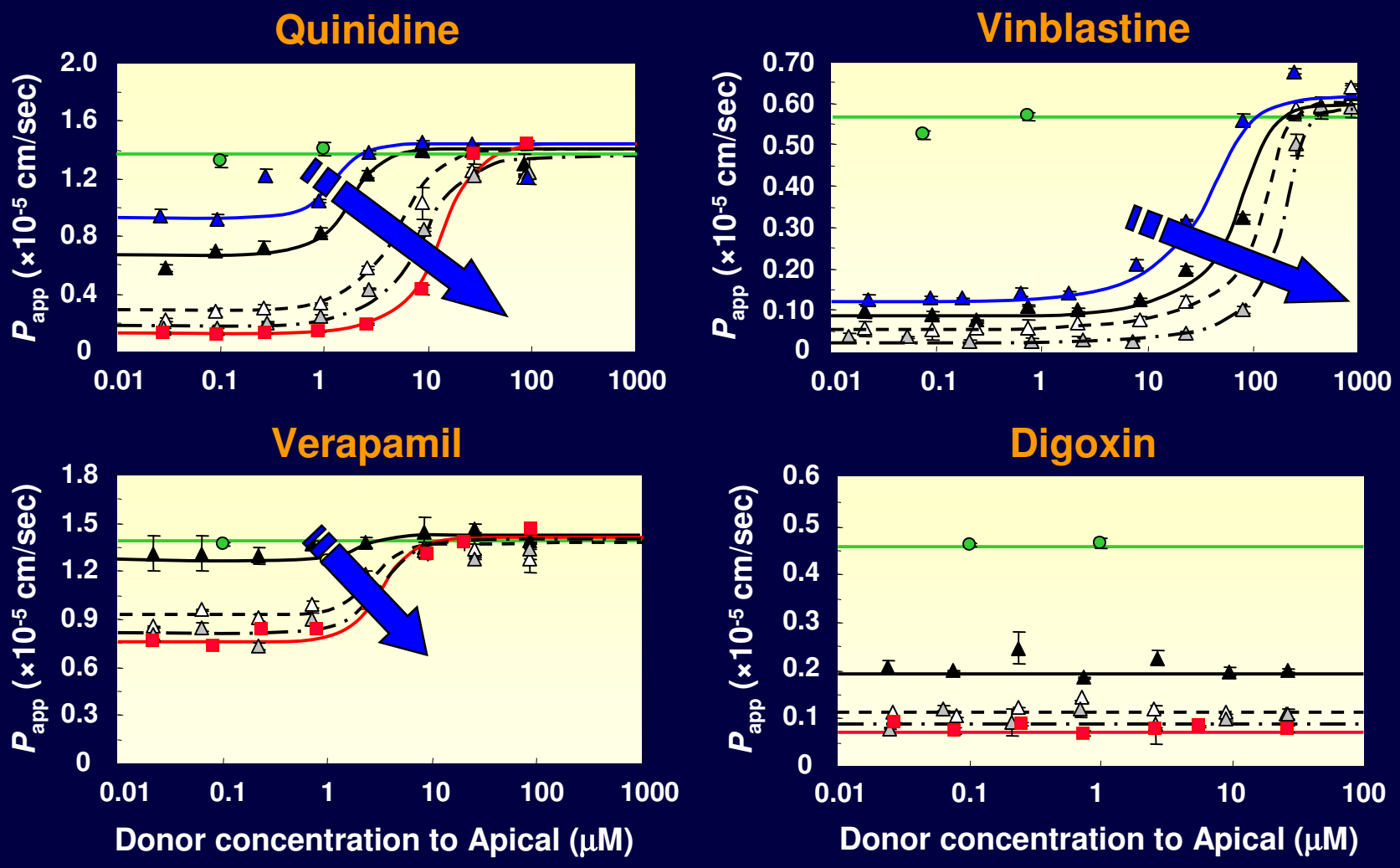
Various cell lines with different expression levels of P-gp



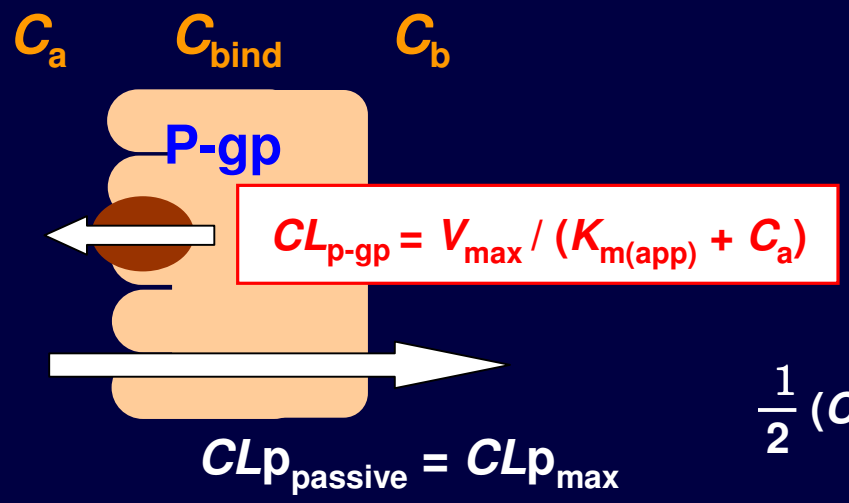
	cell line	P-gp expression levels	
		Protein (μg/cm^2)	mRNA (×10^{-5}/GAPDH)
1	Non-cell (Blank)	0.00	0.00
2	Normal Caco-2 cells	26.8	3.22
3	P-gp-induced Caco-2 cells	103.7	5.20
4	P-gp-highly induced Caco-2 cells	191.0	9.53
5	MDR1-knockdown Caco-2 cells	8.71	1.32
6	MDR1-MDCKII cells	359.6	144.59

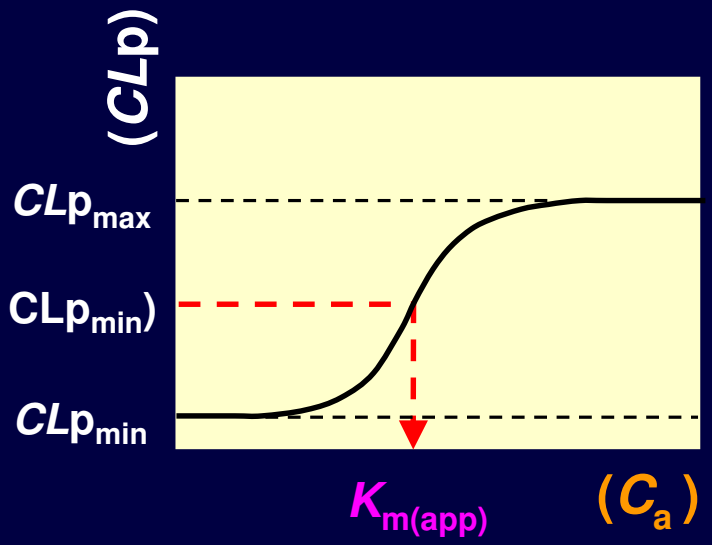
Protein levels were detected and quantified by Western blotting, loading total protein of 50μg for cells and standard.
mRNA levels were determined and quantified by Real-time quantitative PCR.

Effect of donor (apical) concentration on AP to BL transport of P-gp substrates in various cell monolayers



Analysis of P-gp function based on "Sigmoid E_{max} model"





$$CLp = CLp_{max} - CL_{P\text{-}gp}$$

Sigmoid E_{max} model

$$\text{Flux rate} = CLp_{max} \cdot C_a - \frac{V_{max} \cdot C_a^{\,r}}{K_{m(app)}^{\,r} + C_a^{\,r}}$$

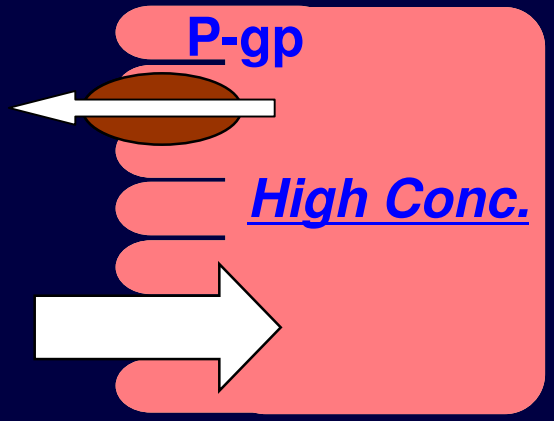
To add the flexibility, Hill Coefficient (r) was introduced to Sigmoid E_{max} model

Estimation of $K_{m(app)}$, V_{max} and hill coefficient of three P-gp substrates by fitting CLp to Sigmoid E_{max} model

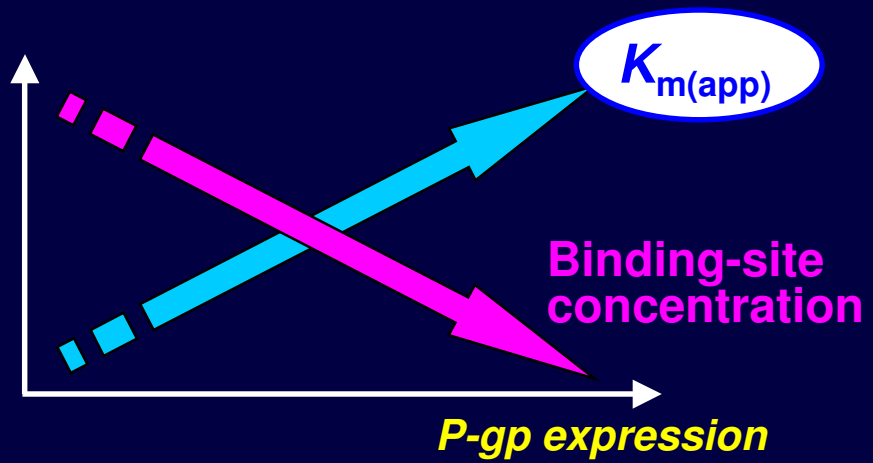
	Cell line	MDR1-kd. Caco-2	Normal Caco-2	P-gp id. Caco-2	P-gp highly id. Caco-2	MDR1-MDCK Ⅱ
	P-gp expression level	→				
Quinidine	$K_{m(app)}$ (μM)	0.61	1.69	6.20	8.13	16.39
	V_{max} ($\times 10^{-7}$ μmol/min/cm^2)	3.00	7.74	41.0	63.0	132.5
	r (Hill coefficient)	1.16	0.98	1.00	1.01	1.02
Verapamil	$K_{m(app)}$ (μM)	—	1.01	1.66	2.07	2.85
	V_{max} ($\times 10^{-7}$ μmol/min/cm^2)	—	0.91	5.09	8.40	12.6
	r (Hill coefficient)	—	1.02	1.00	1.01	1.02
Vinblastine	$K_{m(app)}$ (μM)	29.88	80.69	149.01	323.35	—
	V_{max} ($\times 10^{-7}$ μmol/min/cm^2)	88.3	213.7	498.7	1180.9	—
	r (Hill coefficient)	1.01	1.01	1.00	1.01	—

Why does "$K_{m(app)}$" value fluctuate depending on the expression level of P-gp in cells

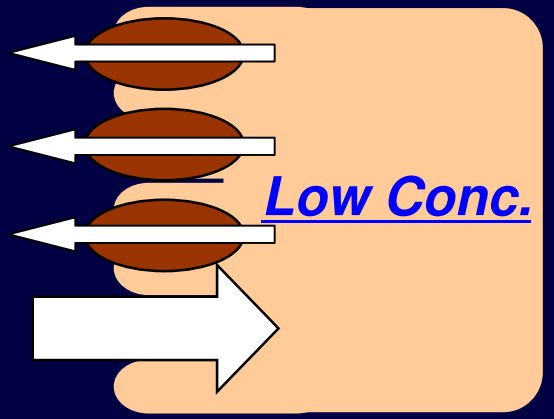
Lower expression level



Drug concentration at drug binding-site of P-gp increase easily when the apical concentration become high.

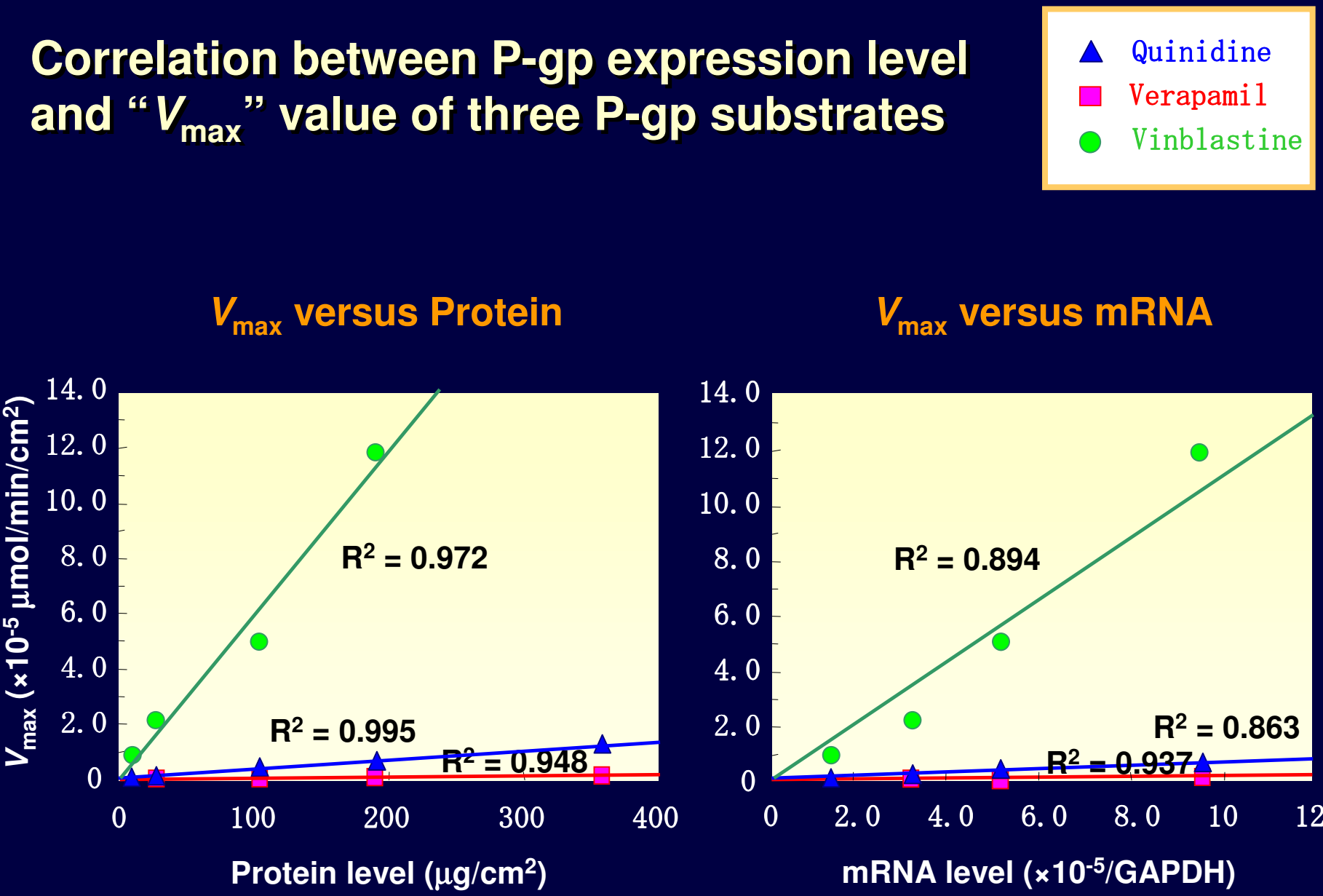


Higher expression level

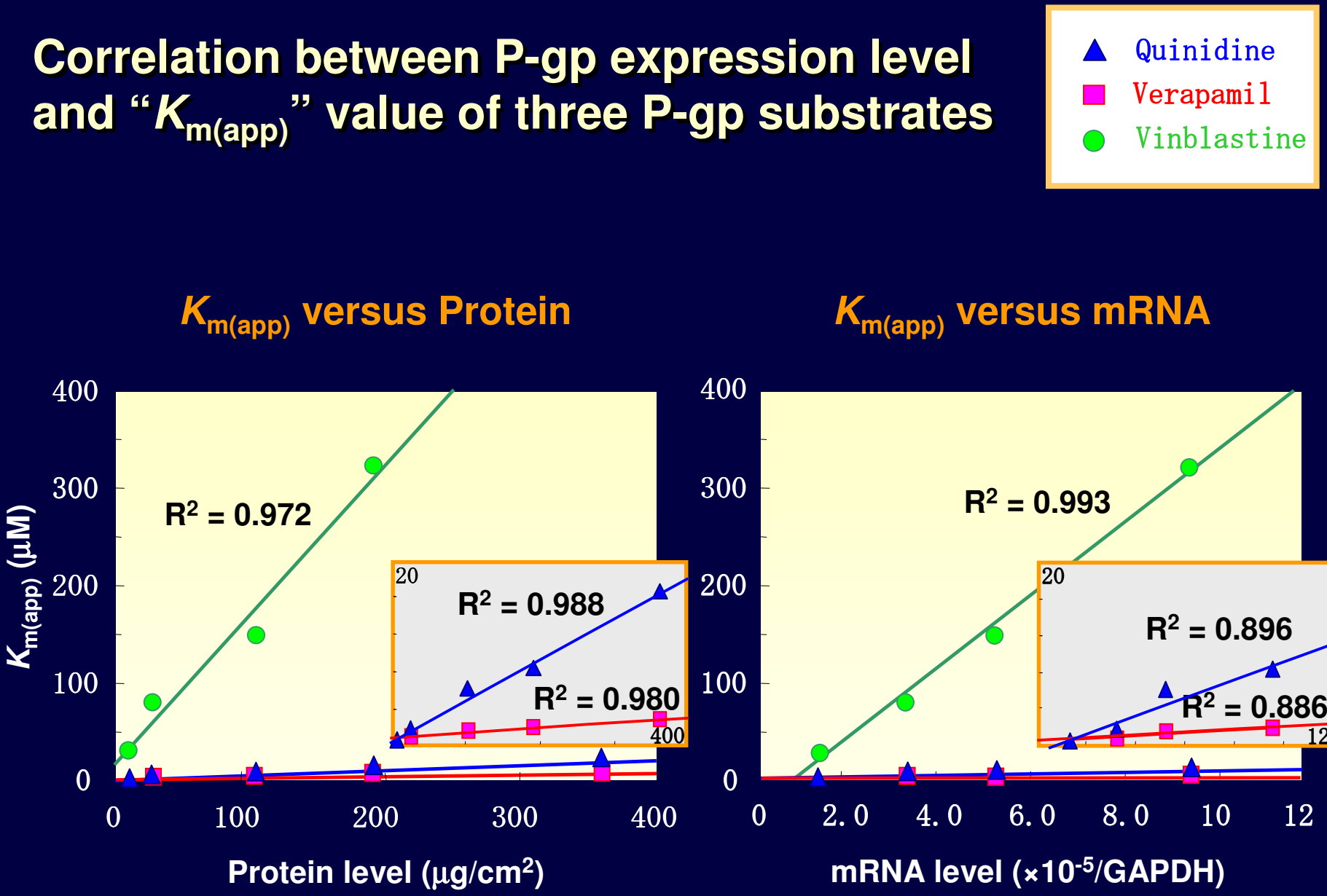


Drug concentration at drug binding-site of P-gp is kept low even when the apical concentration become high.

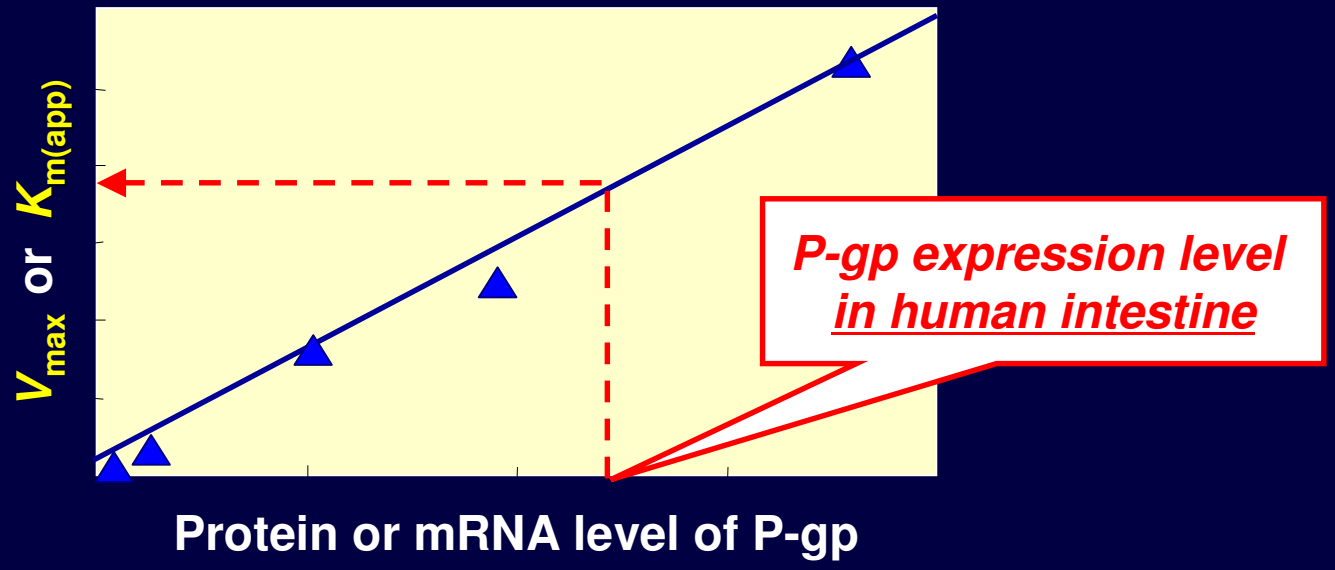
Correlation between P-gp expression level and "V_{max}" value of three P-gp substrates



Correlation between P-gp expression level and "$K_{m(app)}$" value of three P-gp substrates



How to estimate P-gp-mediated efflux in human intestine? How to predict the permeability in human intestine?



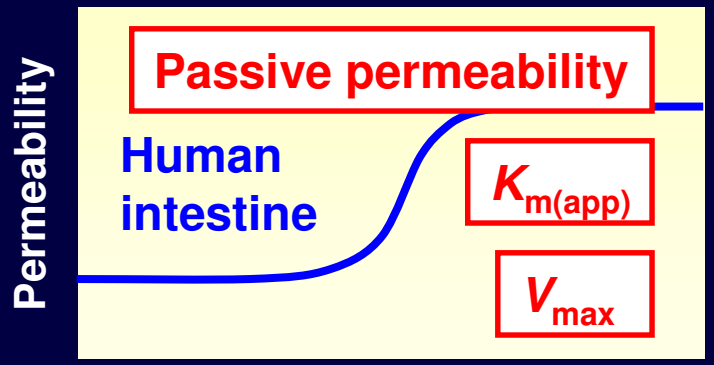
(1) **Passive permeability :**

Possible to be predicted from Caco-2 data by using P-gp inhibitor.

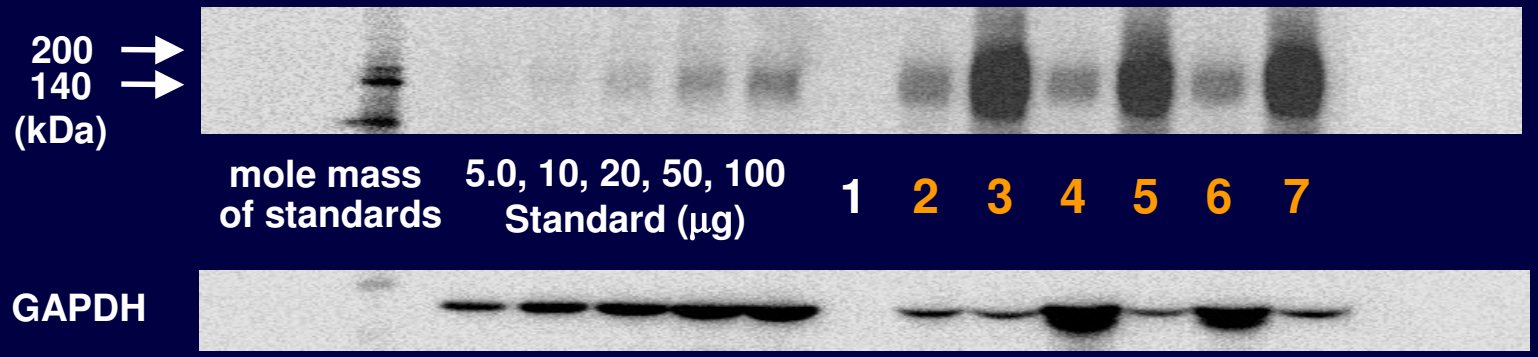
(2) **P-gp mediated efflux :**

$CL_{p\text{-}gp} = V_{max} / (K_{m(app)} + C_a)$

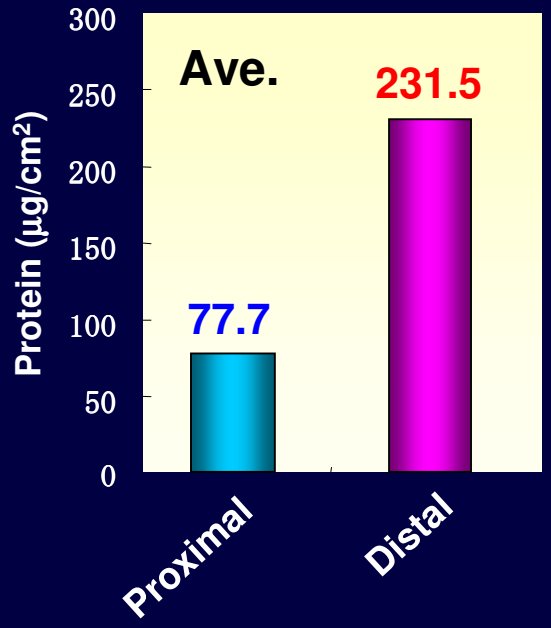
***In vivo* permeability**



Regional difference in P-gp expression levels in rat small intestine (proximal and distal)

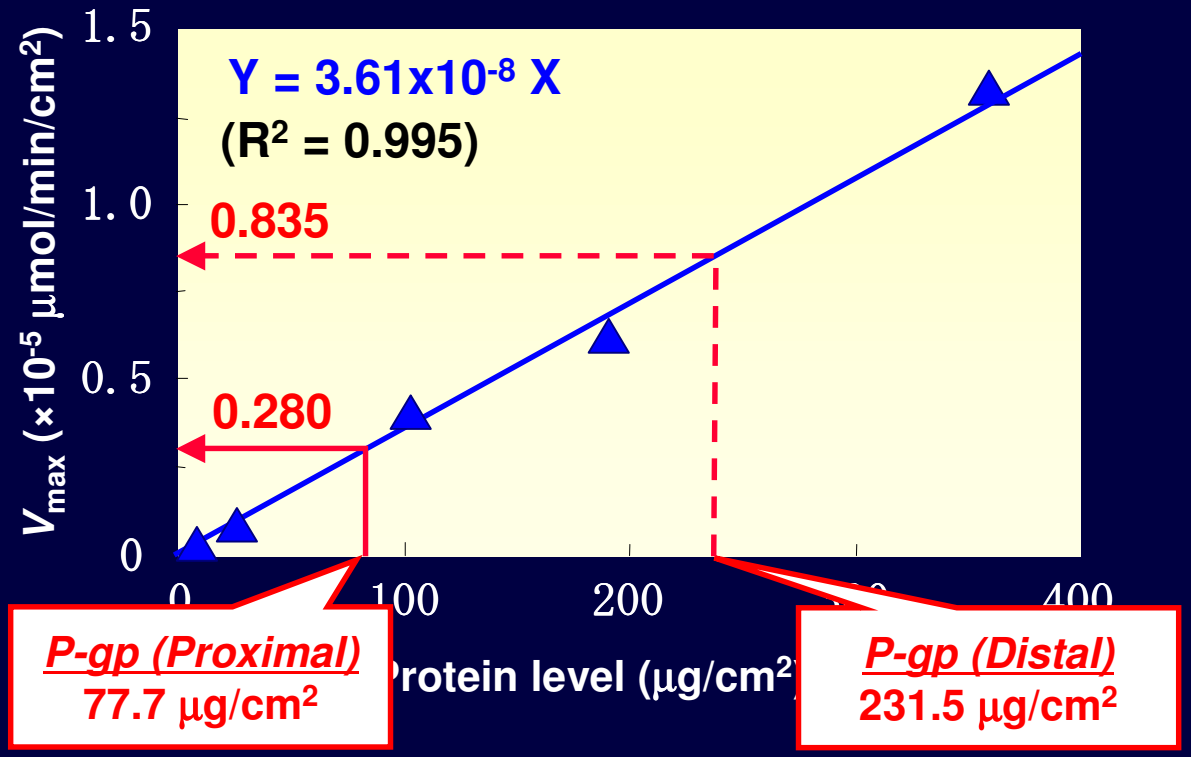


Rat # / Region			P-gp expression levels Protein (μg/cm²)
Rat #1	1	Blank	0.00
	2	Proximal	89.8
	3	Distal	291.7
Rat #2	4	Proximal	65.7
	5	Distal	166.8
Rat #3	6	Proximal	77.6
	7	Distal	235.9



Protein levels were detected and quantified by Western blotting, loading 100μg for rat BBM; 50μg for standard.

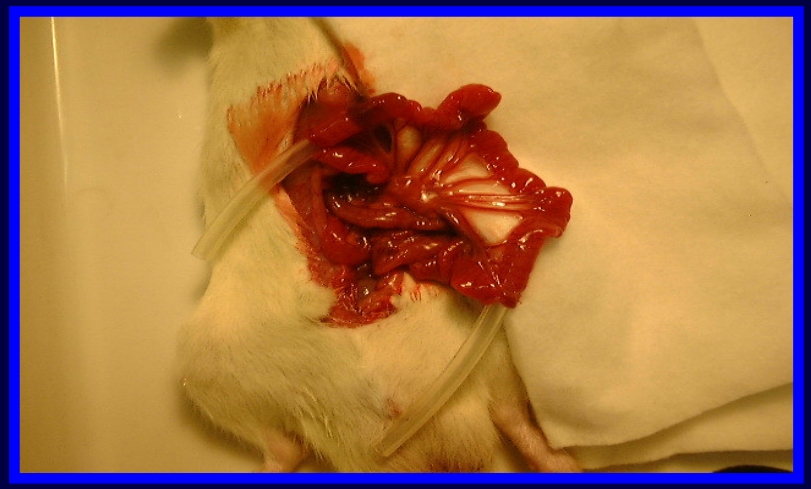
Estimation of "V_{max}" and "$K_{m(app)}$" values of quinidine in rat small intestine (proximal and distal)



Quinidine		V_{max} (×10^{-5} μmol/min/cm^2)	$K_{m(app)}$ (μM)
Rat intestine	Proximal	*0.280*	*1.615*
	Distal	*0.835*	*3.202*

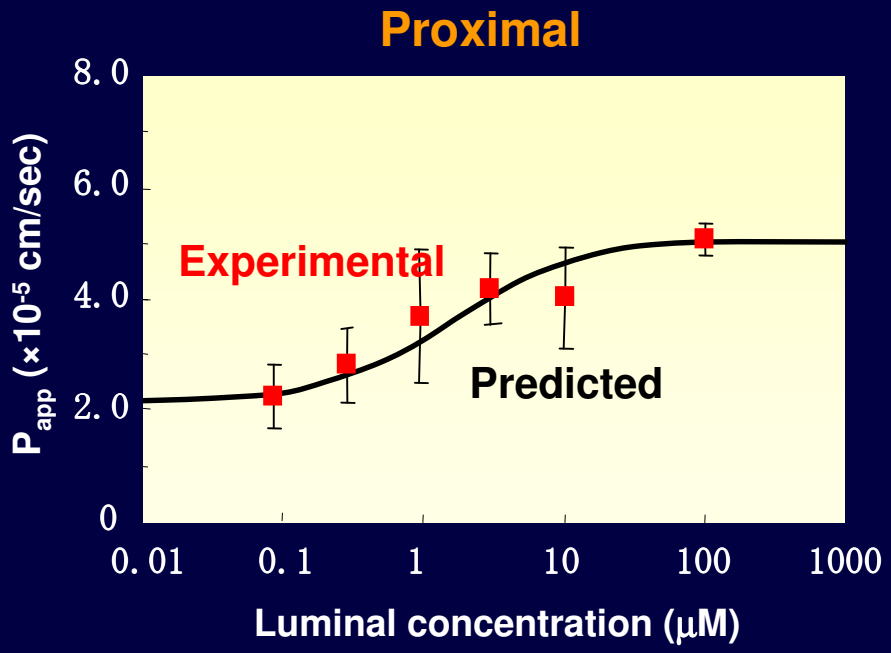
Passive permeability "P_{app}" of quinidine in rat small intestine (proximal and distal)

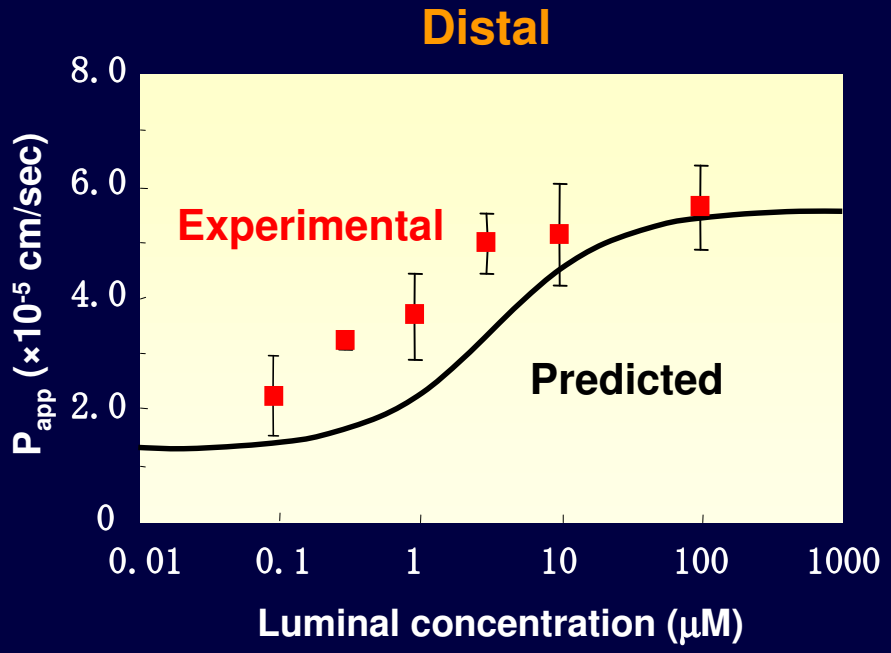
		Quinidine	
		Passive P_{app} ($\times 10^{-5}$ cm/sec)	Ratio (Rat/Caco-2)
Cell monolayers		*1.41*	—
Rat intestine	Proximal	*5.06*	3.59
	Distal	*5.60*	3.97



P_{app} of quinidine to rat small intestine was measured by *in situ* single-pass perfusion experiments.

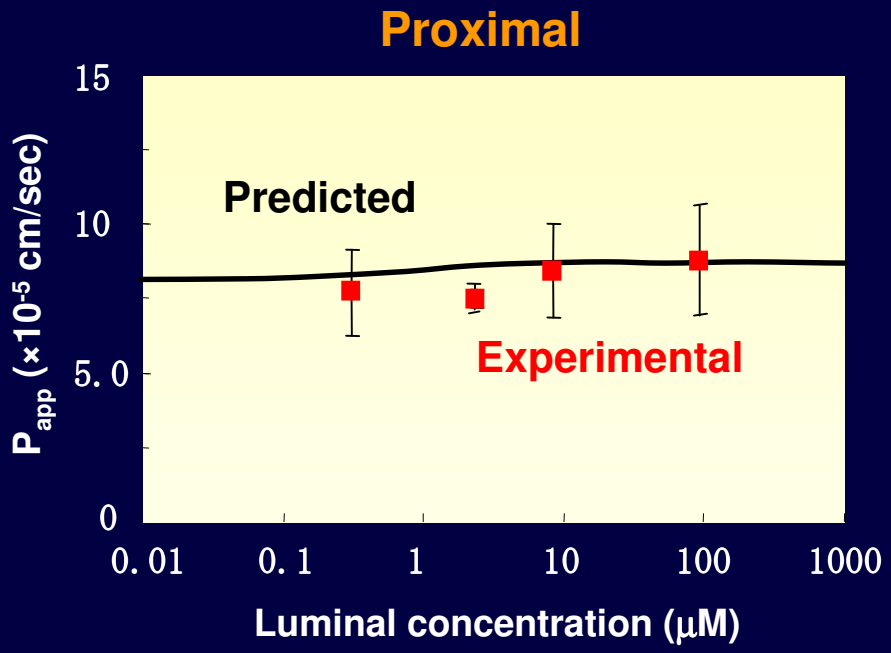
Simulation of concentration-dependent permeability of quinidine in rat small intestine (proximal and distal)

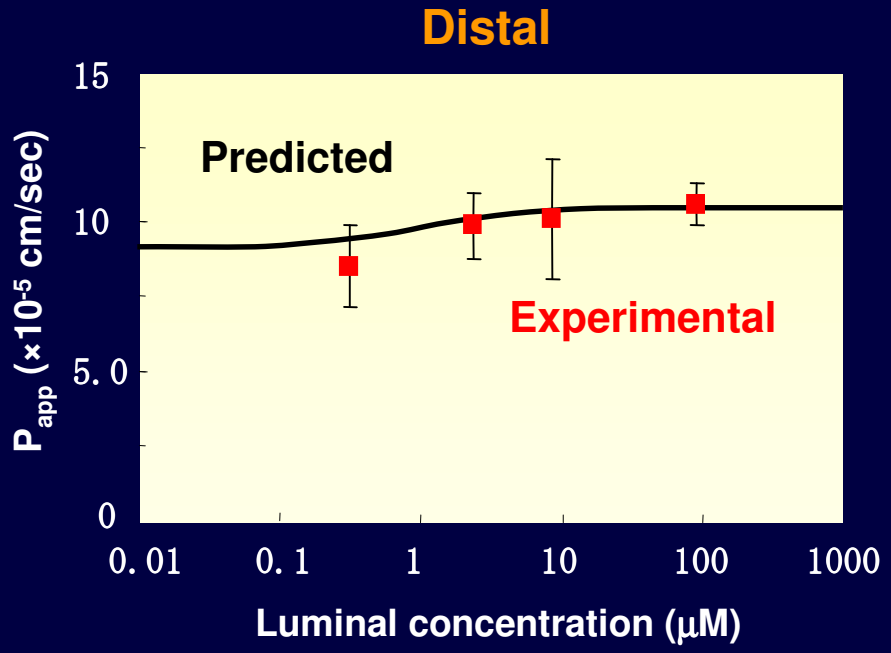




	Proximal			Distal		
	V_{max} (x10^{-5} μmol/min/10cm gut)	$K_{m(app)}$ (μM)	$V_{max}/K_{m(app)}$ (x10^{-5} L/min /10cm gut)	V_{max} (x10^{-5} μmol/min/10cm gut)	$K_{m(app)}$ (μM)	$V_{max}/K_{m(app)}$ (x10^{-5} L/min /10cm gut)
Predicted	*3.13*	*1.61*	*1.94*	*9.35*	*3.20*	*2.92*
Experimental	*1.08*	*0.60*	*1.80*	*1.70*	*0.70*	*2.42*

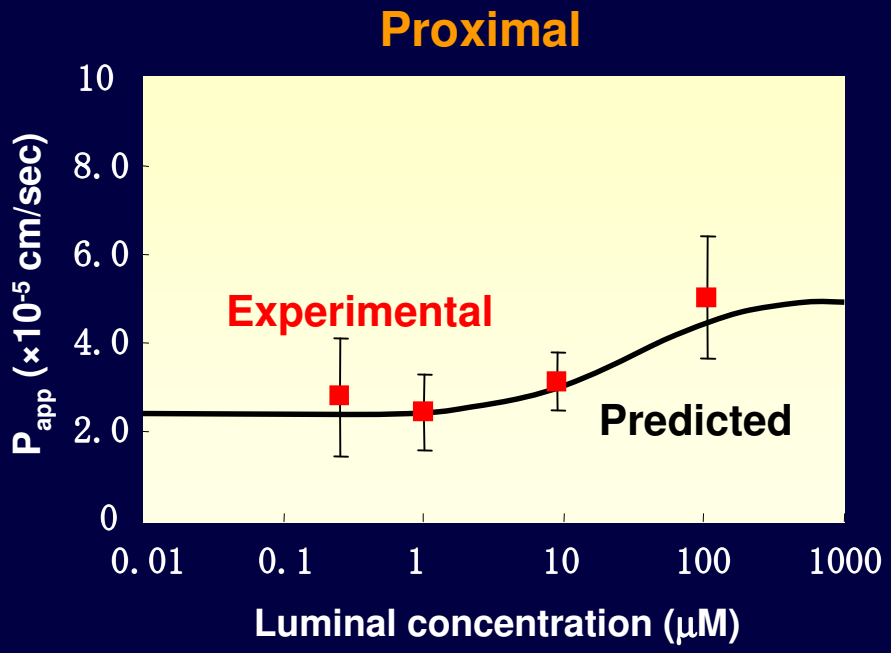
Simulation of concentration-dependent permeability of verapamil in rat small intestine (proximal and distal)

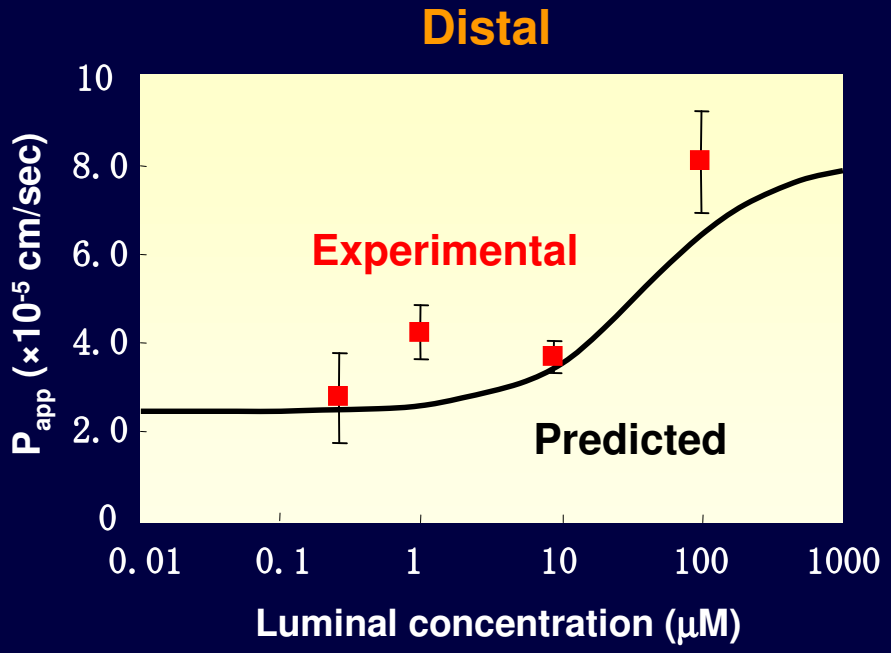




	Proximal			Distal		
	V_{max} (×10^{-5} μmol/min/10cm gut)	$K_{m(app)}$ (μM)	$V_{max}/K_{m(app)}$ (×10^{-5} L/min /10cm gut)	V_{max} (×10^{-5} μmol/min/10cm gut)	$K_{m(app)}$ (μM)	$V_{max}/K_{m(app)}$ (×10^{-5} L/min /10cm gut)
Predicted	*0.33*	*0.92*	*0.36*	*0.98*	*1.02*	*0.95*
Experimental	*0.69*	*0.87*	*0.80*	*1.50*	*0.82*	*1.83*

Simulation of concentration-dependent permeability of vinblastine in rat small intestine (proximal and distal)



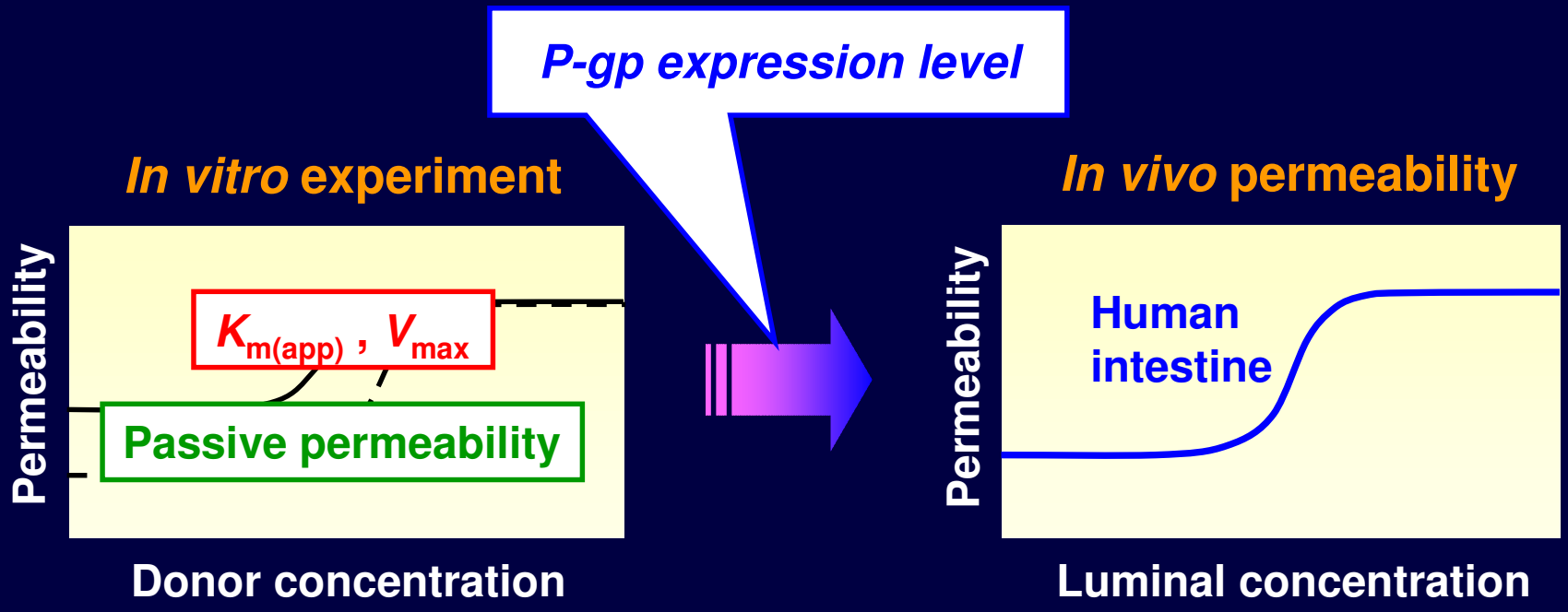


	Proximal			Distal		
	V_{max} (×10^{-5} μmol/min/10cm gut)	$K_{m(app)}$ (μM)	$V_{max}/K_{m(app)}$ (×10^{-5} L/min /10cm gut)	V_{max} (×10^{-5} μmol/min/10cm gut)	$K_{m(app)}$ (μM)	$V_{max}/K_{m(app)}$ (×10^{-5} L/min /10cm gut)
Predicted	*51.3*	*29.3*	*1.75*	*153*	*40.9*	*3.73*
Experimental	*38.6*	*23.1*	*1.67*	*111*	*32.9*	*3.37*

CONCLUSIONS

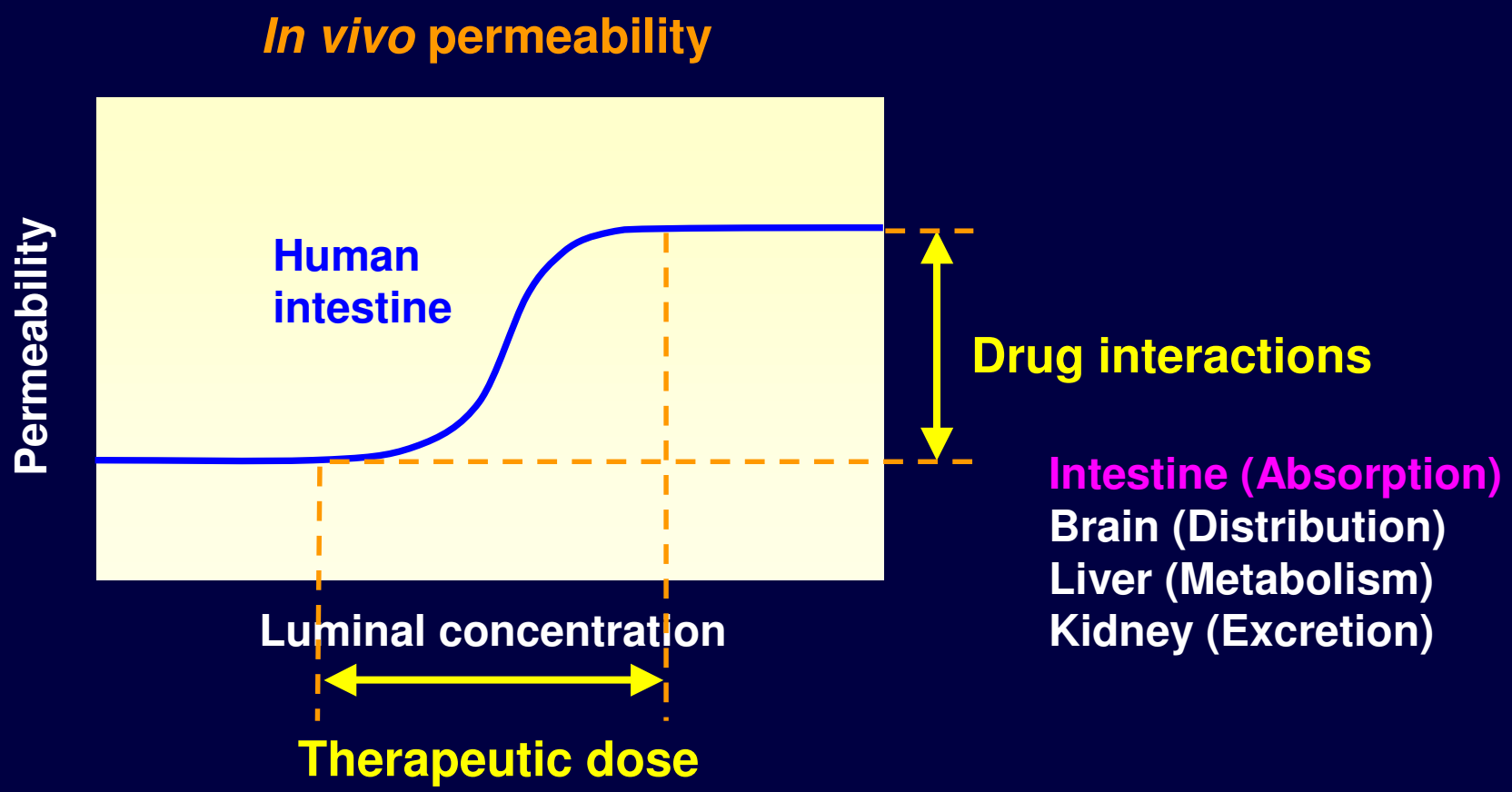
"Sigmoid E_{max} model" for permeability-concentration curve of P-gp substrate drugs can provide the fundamental parameters of P-gp-mediated transport, $K_{m(app)}$ and V_{max}.

This study clearly demonstrated the possibility to estimate $K_{m(app)}$ and V_{max} values in human intestine from P-gp expression level and to predict concentration-dependent permeability of P-gp substrate drugs on human intestinal absorption.



SIGNIFICANCE

Permeability-concentration curve of P-gp substrate drugs can predict how P-gp affect the pharmacokinetic profiles of its drugs and how much permeability of its drugs will be changed by dose adjustment and drug-drug interactions in clinical treatment.



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