26th JSSX Annual Meeting in Hiroshima
November 17th, 2011

for JSSX Award 2011

Prediction and Improvement of Oral Drug Absorption - Comprehensive Study from Cells to Human -

医薬品の消化管吸収に関する in vitroからヒト臨床に至る網羅的研究

Shinji Yamashita, Ph.D.

Setsunan University, Osaka, Japan

26th JSSX Annual Meeting in Hiroshima
November 17th, 2011

for JSSX Award 2011

My Boss

Prof. Hitoshi Sezaki
(Kyoto University, Setsunan U

Prof. Tanskazu Nadai
(Jyosai University, Setsunan

Prof. Toshikiro Kimura
(Kyoto University)

Prof. Gordon L. Amidon
(University of Michigan)

26th JSSX Annual Meeting in Hiroshima
November 17th, 2011

for JSSX Award 2011

Prof. Yuichi Sugiyama (The University of Tokyo)

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“ お

…and further

why same shirt ?

“Comprehensive Study” from Cells to Human

Information required for development of oral drug products

Compound

Absorption potential

- permeability
- solubility
- stability
- DDI (*in vitro*)
- crystal form
- salt formation

Formulation design

Absorption profile

- bioavailability
- dissolution profile
- animal-scale up
  - dose-linearity
  - food effect
  - DDI

Product

Human PK profile

- dose-linearity
- food effect
- DDI
- bioequivalence

Consortium of Oral Drug Absorption Screening (2001 ~ 2009)

Purposes

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4. Es
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Academia

Exchange information

Industry

Platform for researcher

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Activities of the Consortium (2001-2009)

Projects

Project 1: **Standardization and human scale-up of Caco-2 data**

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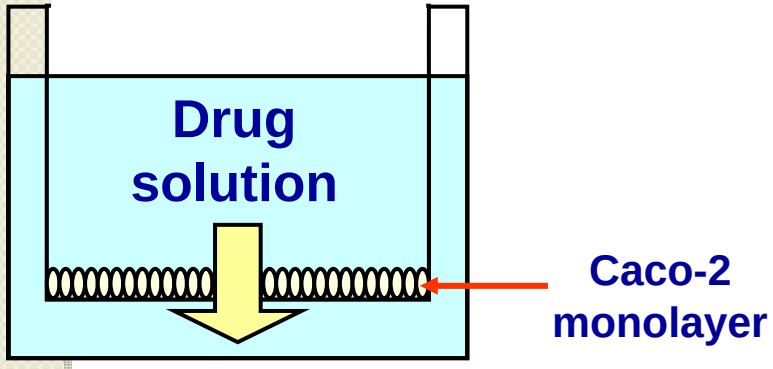
Project 5: **BCS Classification and Oral absorption**

Caco-2 monolayer used for drug permeation assay

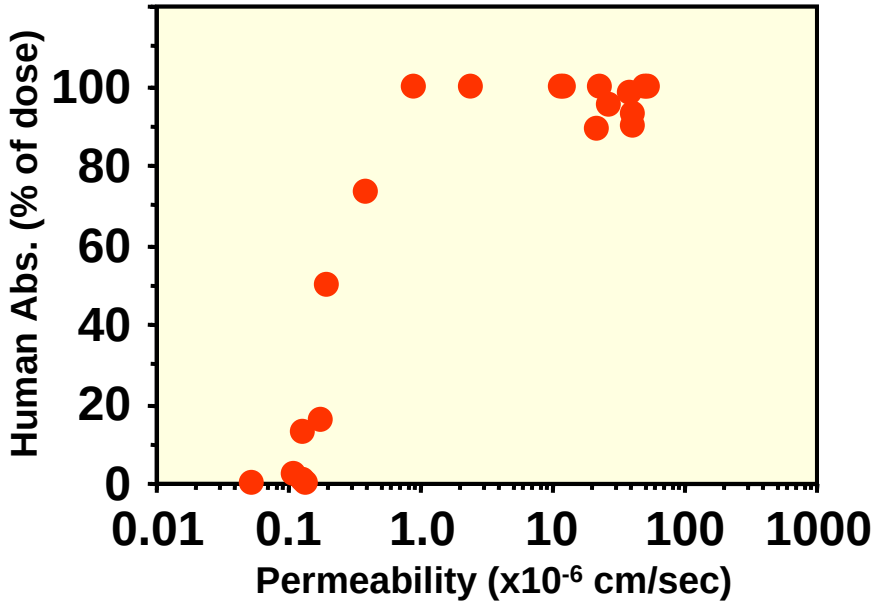
Origin: Colon carcinoma (Human)

Monolayer: 16-21 days culture

Permeability of highly soluble drugs across Caco-2 monolayer well correlates to human absorption.

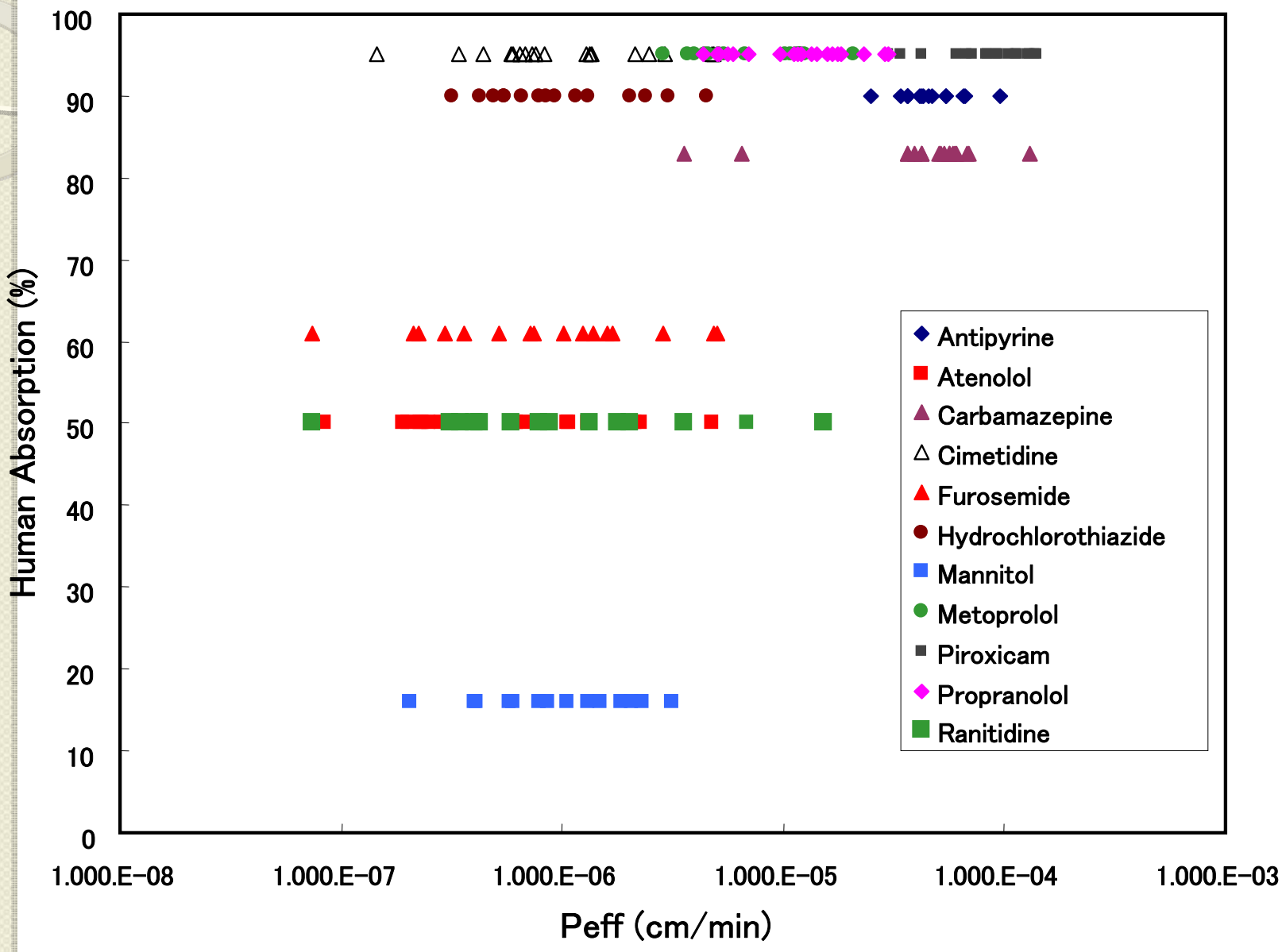


Permeation assay



Data taken from Artursson P et al., *Biochem Biophys Res Commun*, **175**, 880-885 (1991).

Project 1:
Deviation of Peff in Caco-2 study among 20 companies



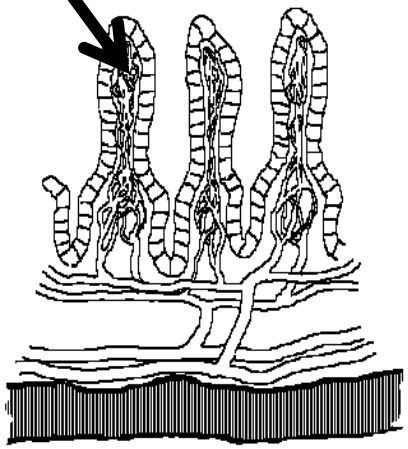
Reasons of Difference in Passive Permeability

$$P_{eff} = P_{trans} + P_{para}$$

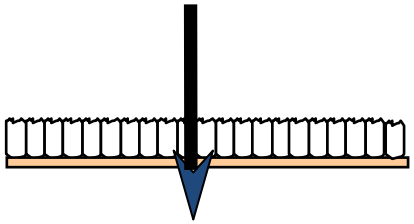
Difference in effective surface area

Difference in tight junction

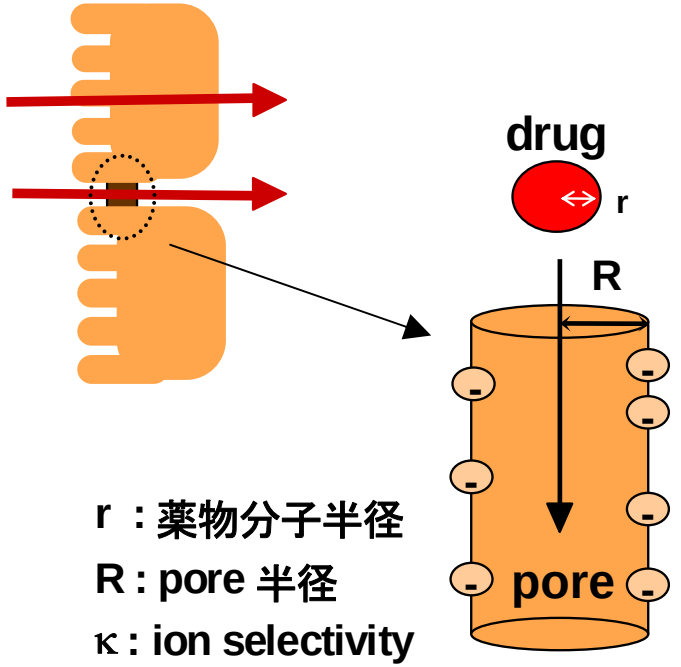
In vivo absorption



In vitro permeation



Intestinal membrane Caco-2 monolayer



r ：薬物分子半径
R : pore 半径
κ : ion selectivity

$$P_{trans} = A \times P_{intrinsic}$$

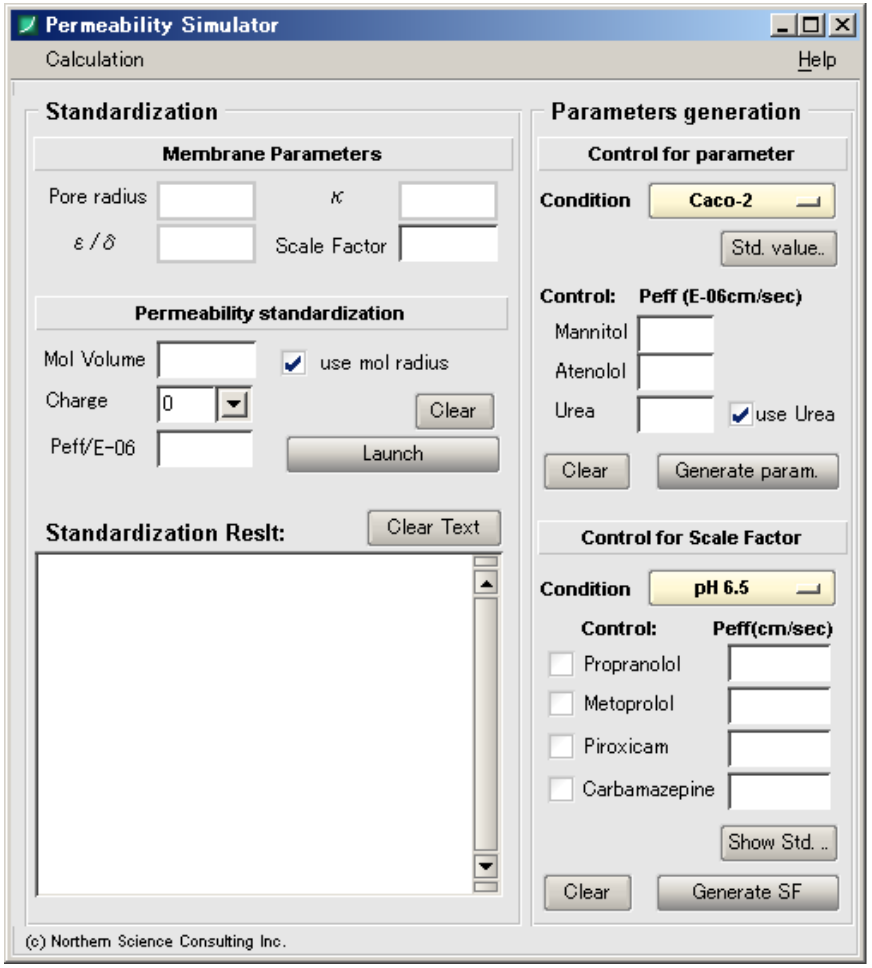
Instruction manual of PermeSimulator

2005.07.29 Northern Science Consulting Inc.

下の説明にある、①、②の順序でPeffの実測値を入力してパラメータを生成します。算出されたパラメータがパラメータ表示部に示され、それらを用いて③で化合物データを入力し標準化します。結果は、Stanrdization Result:に表示されます。

数値を変えて結果の変化を見るようなテスト画面と終了のメニューです。

プログラムで使われる定数、基本パラメータとバージョンが表示されます。



パラメータ表示部

③標準化する

化合物のモル体積、チャージ、Peff値を入力し、Launchをクリックします。複数の化合物についてはこの操作を繰り返します。

①Pore半径、ε / δ 、κを得る

Mannitol, Atenolol, Urea の実測Peff値を入力します。Ureaが得られないときはチェックをはずします。Generate param. をクリックするとウインドウ左上部のMembrane Parameters に値がセットされます。

②Scale Factorを得る

使用するControlをチェックし、各ControlのPeff値を入力します。値が入力され、チェックされたものだけが使用されます。Generate SFをクリックするとウインドウ左上部のMembrane ParametersのScale Factorに値がセットされます。
注）Scale Factor値は、パラメータ表示部での直接入力も可能です。

他のボタン説明

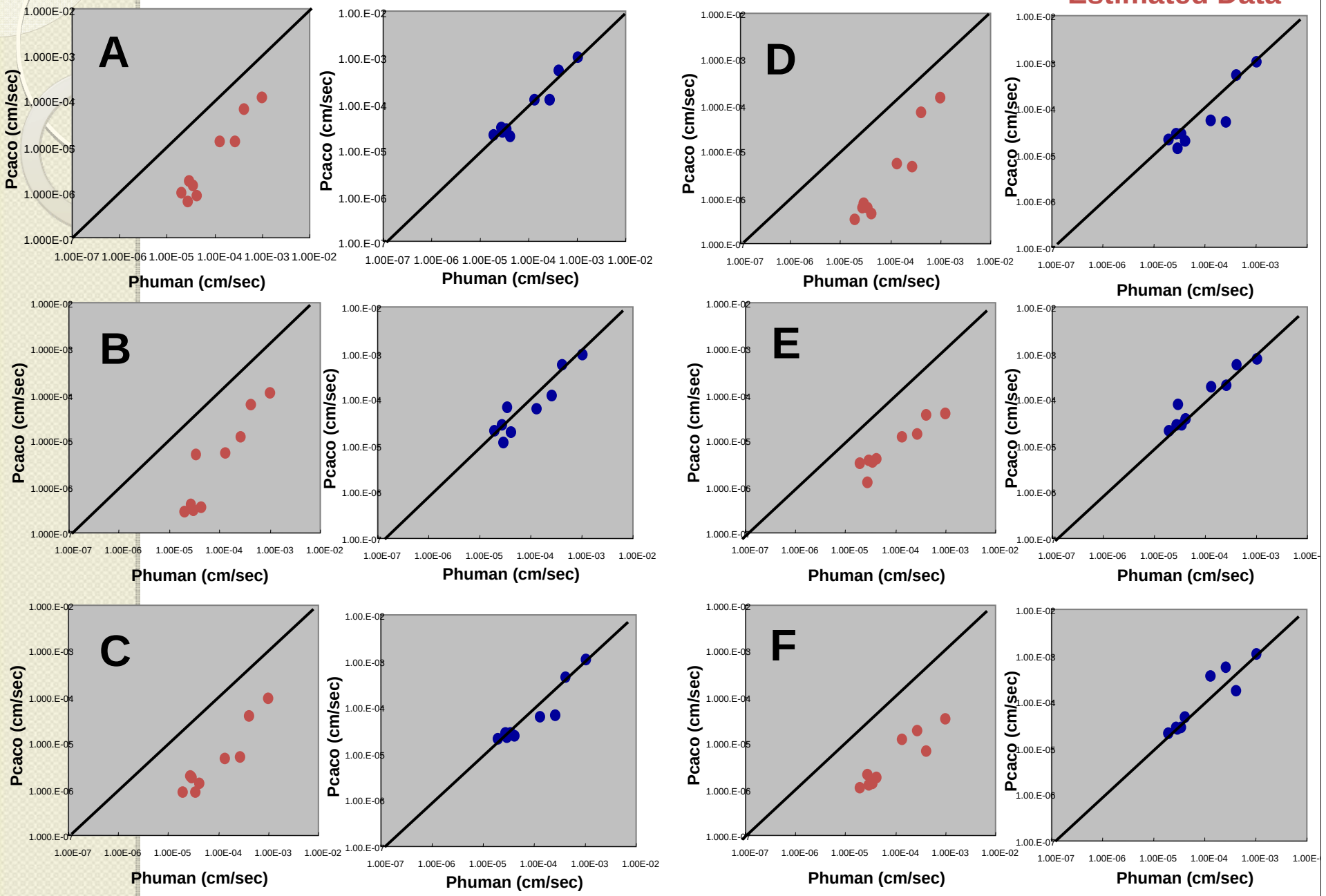
Clear 各入力エリアをクリアします。　Std. value 膜の基準値を表示します。　Show Std. SFの基準値を表示します。

Raw data

Estimated Data

Raw data

Estimated Data



Activities of the Consortium (2001-2009)

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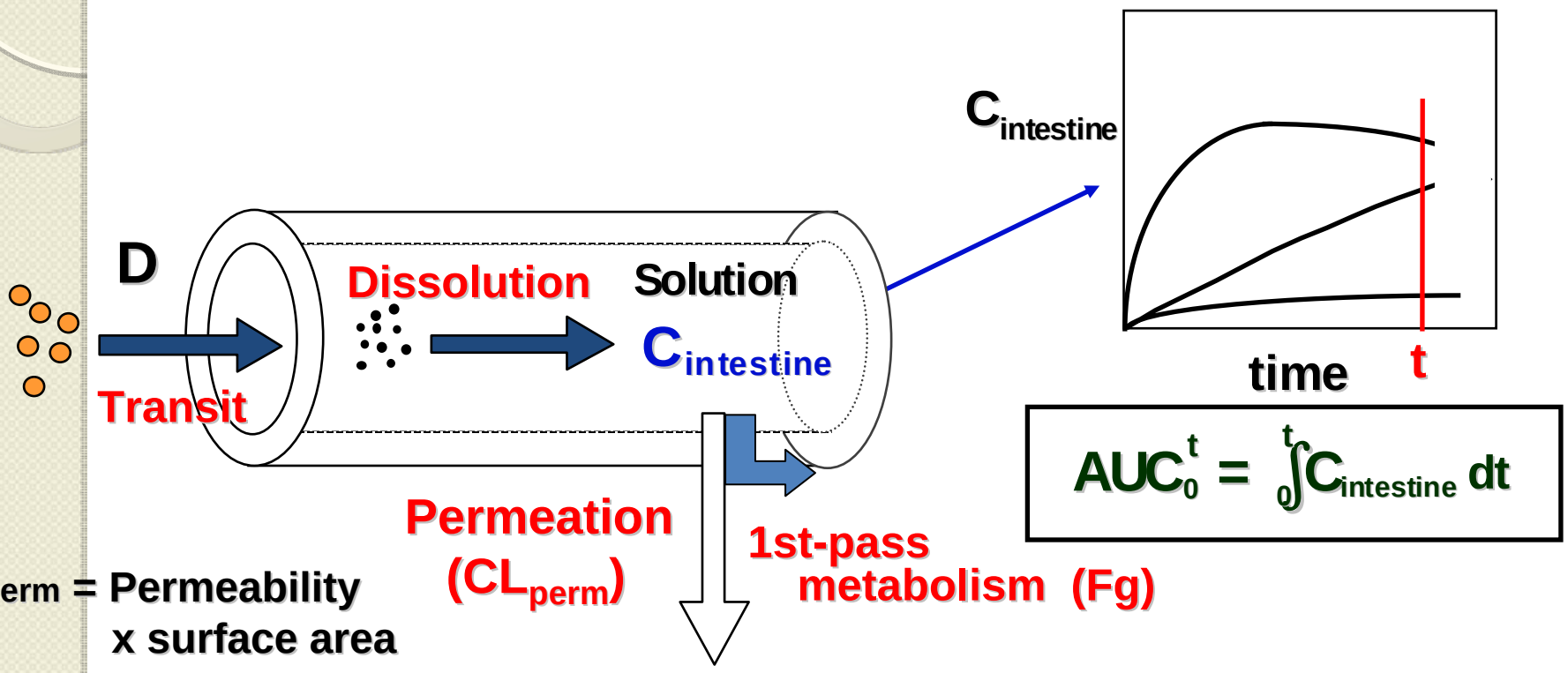
Project 5: **BCS Classification and Oral absorption**

Macroscopic analysis of drug absorption

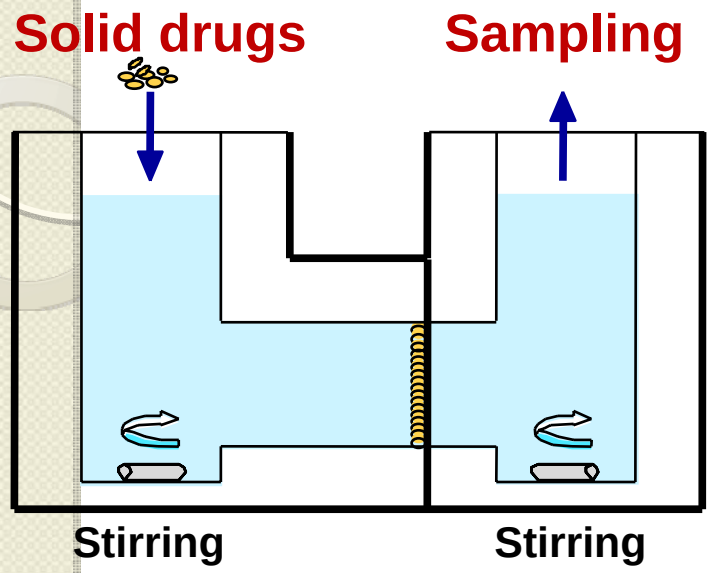


$$AUC_0^t = \int_0^t C_{intestine}\, dt$$

CL_{perm} = Permeability x surface area

$$\text{Absorbed amount} = \underbrace{AUC_0^t \times CL_{perm}}_{Fa} \times Fg$$

In vitro System - Dissolution/Permeation system



Fluid volume

Human*

500 - 1000 mL

D/P system

8.0 mL

*Cited from Dressman JB et al., *Eur J Pharm Sci*, **11** Suppl 2, S73-80 (2000).

	Apical side	Basal side
Volume	8.0 mL	5.5 mL
pH	6.5	7.4
Medium	Simulated intestinal fluid	4.5 w/v% BSA
Stirring rate	200 rpm	200 rpm
Applied amount	1/100 of clinical dose	—

[a] TM: HBSS supplemented with 19.45 mM glucose and 10 mM HEPES.

Kataoka M, *Masaoka Y, Yamazaki Y, Sakane T, Sezaki H, Yamashita S Pharm Res.* ***20(10)****:1674-80 .(2003)*

Estimation of food-effect on oral absorption of albendazole, quazepam and nateglinide from in vitro study in D/P system

Drug & Food state	D/P system				*In vivo*
	Applied amount (mg)	Permeated amount (%)	Estimated absorption (%)		AUC ratio (Fasted/Fed)
Albendazole (strongly affected)					
Fasted state	4.0	0.041	14	3.5	4.0
Fed state		0.122	49		
Quazepam (slightly affected)					
Fasted state	0.2	1.174	75	1.2	1.6
Fed state		0.778	91		
Nateglinide (not affected)					
Fasted state	0.6	14.712	97	1.0	0.9
Fed state		11.109	100		

† Permeated amount (% of dose/2 h)

‡ Predicted absorption was calculated by Eq. 2

Prediction of formulation-effect on oral absorption of danazol from in vitro study in D/P system

Dosage form & Food state	D/P system: Applied amount (mg)	D/P system: Permeated amount (% of dose/2 h)	D/P system: Estimated absorption (% of dose)	*In vivo*: Human Absorption (%)
Crude powder				
Fasted state	1.0	0.125 ±0.003	30	24
Fed state		0.250 ±0.006	71	76
Gelucire 44/14				
Fasted state	1.0	1.101 ±0.089	74	92
Fed state		1.787 ±0.078	97	100

Kataoka M, *Masaoka Y, Sakuma S, Yamashita S*
J Pharm Sci. ***95(9)****:2051-61. (2006)*

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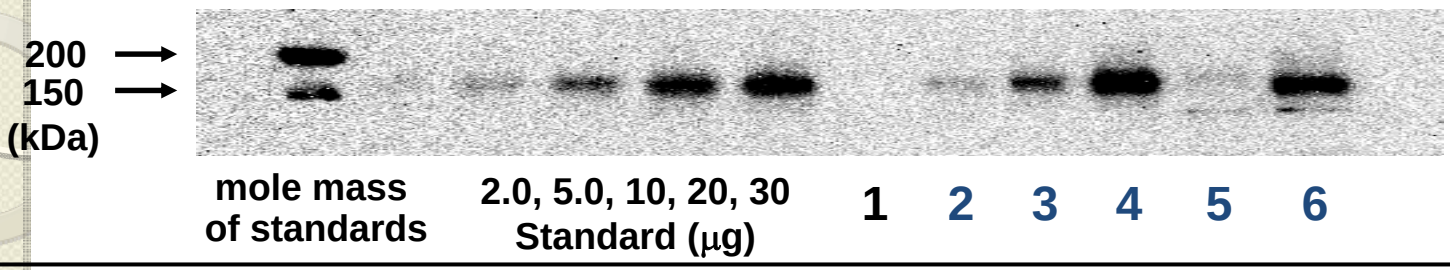
Cell lines having different levels of P-gp expression

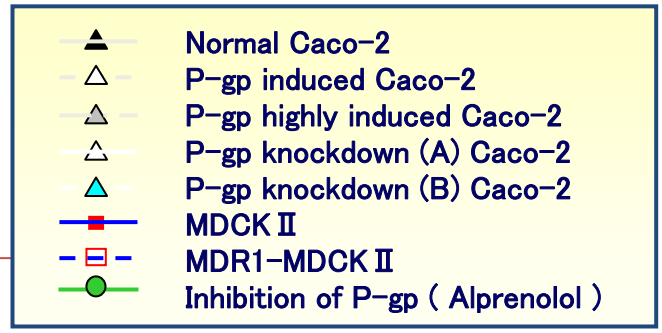


	cell line	P-gp expression levels	
		Protein (/cm^2)	mRNA (×10^{-2}/GAPDH)
1	Non-cell (Blank)	0.00	0.00
2	Normal Caco-2 cells	13.8	3.14
3	P-gp induced Caco-2 cells	113.2	5.11
4	P-gp highly induced Caco-2 cells	344.4	10.42
—	P-gp knockdown (A) Caco-2 cells	—	1.32
—	P-gp knockdown (B) Caco-2 cells	—	0.076
5	MDCKⅡ cells	9.20	0.066
6	MDR1-MDCKⅡ cells	506.2	142.99

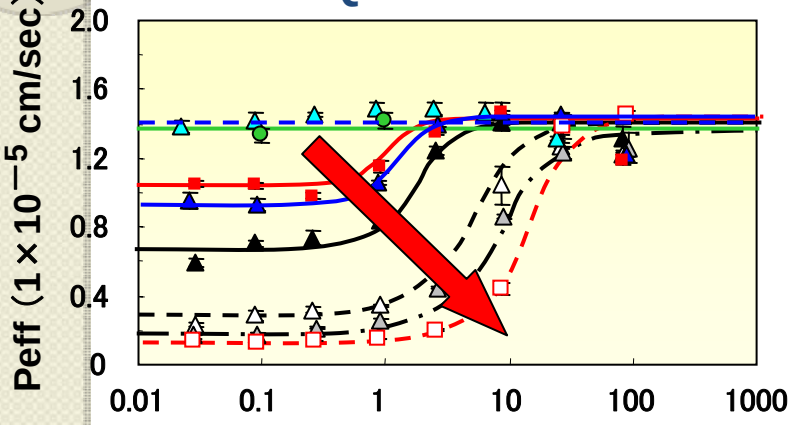
Protein levels were detected by Western blotting, quantified with computer fluorescence analysis. mRNA levels were determined and quantified by Real-time quantitative PCR.

Shirasaka Y, *Masaoka Y, Kataoka M, Sakuma S, Yamashita S. Drug Metab Dispos.* ***36(5)****:916-22. (2008)*

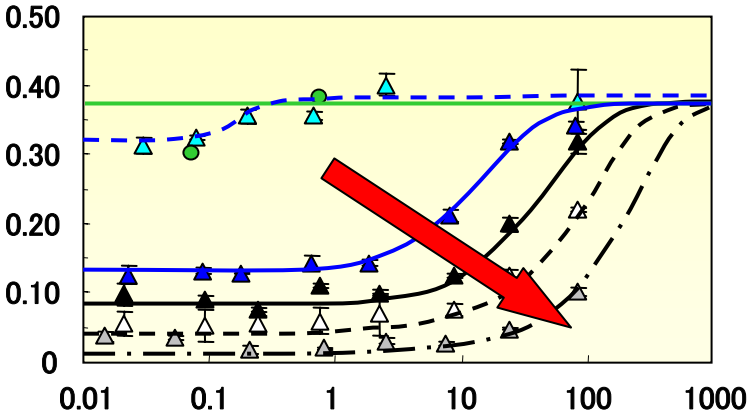
Effect of donor (Apical) concentration on AP to BL transport of P-gp substrates in various cell monolayers



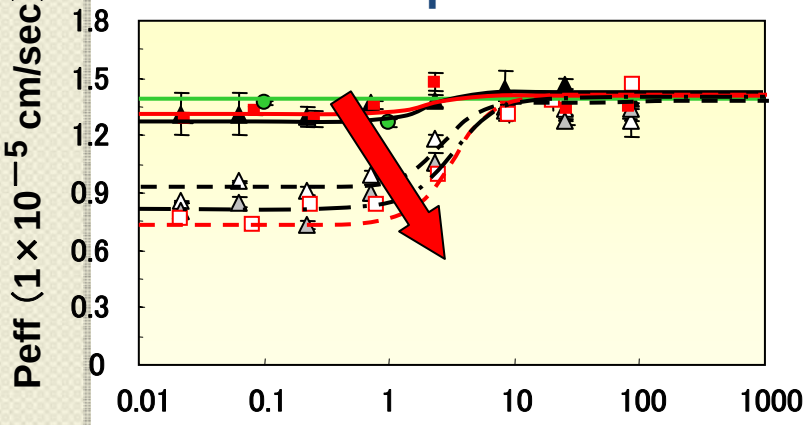
Quinidine



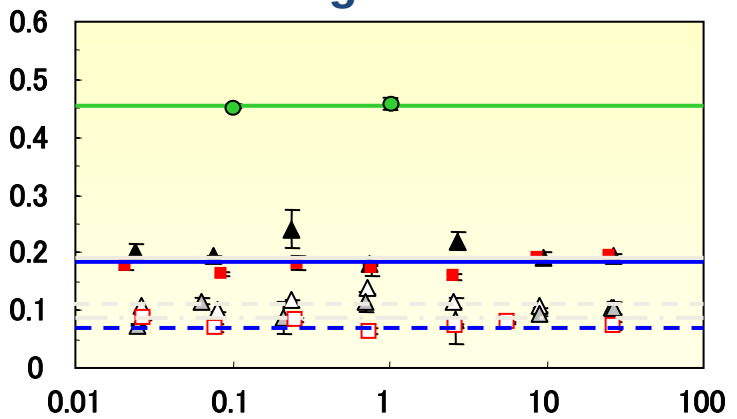
Vinblastine



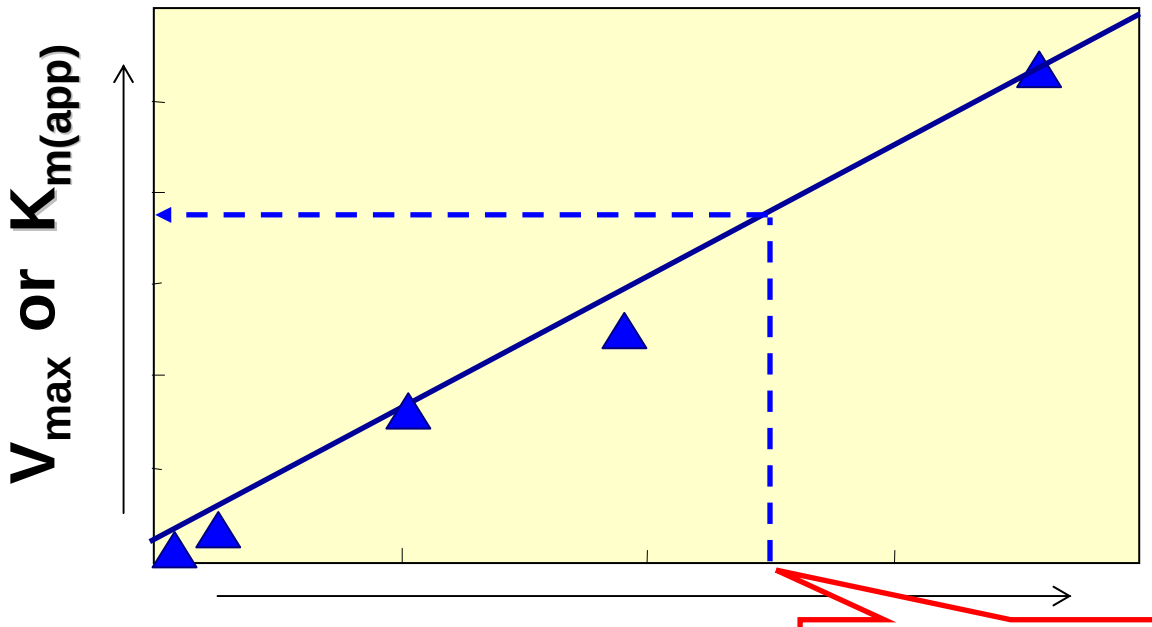
Verapamil



Digoxin



Estimation of P-gp mediated efflux in human intestine



1. P-gp mediated efflux :

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

2. Passive permeability :

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

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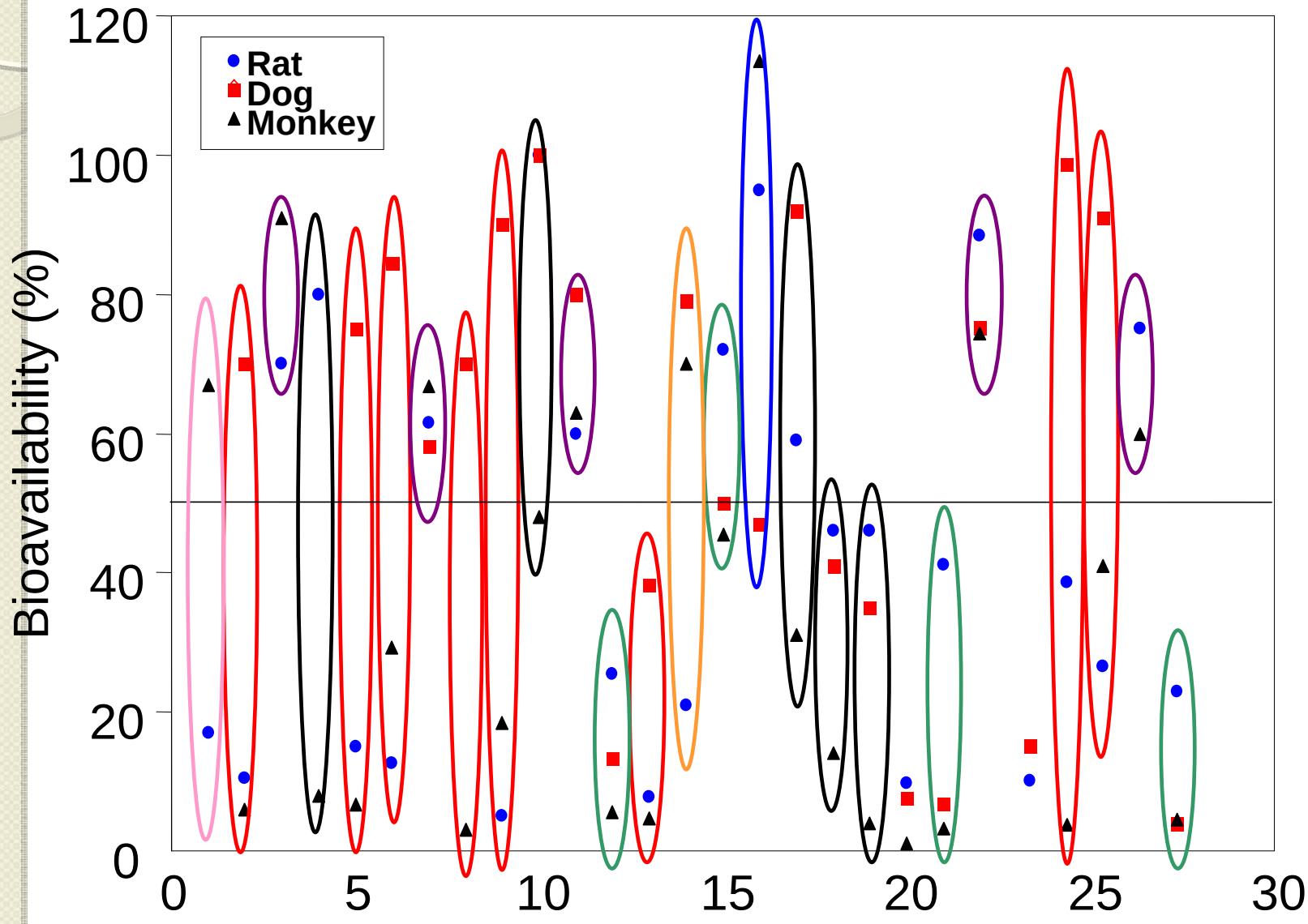
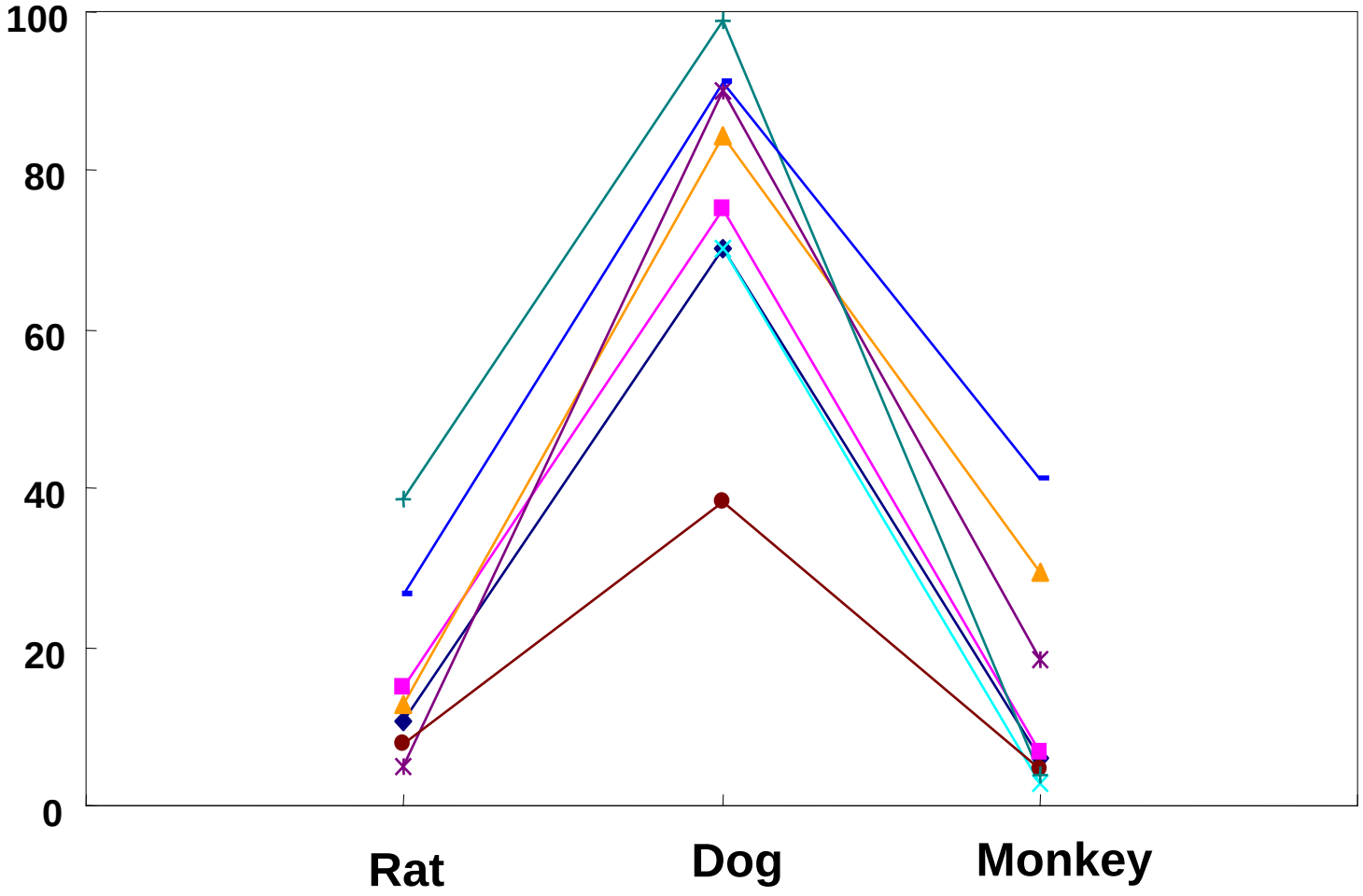
Bioavailability of 27 drug candidates in Japanese pharmaceutical companies



Most frequent pattern of species difference in BA

BA

High BA only in Dog (8 / 27)

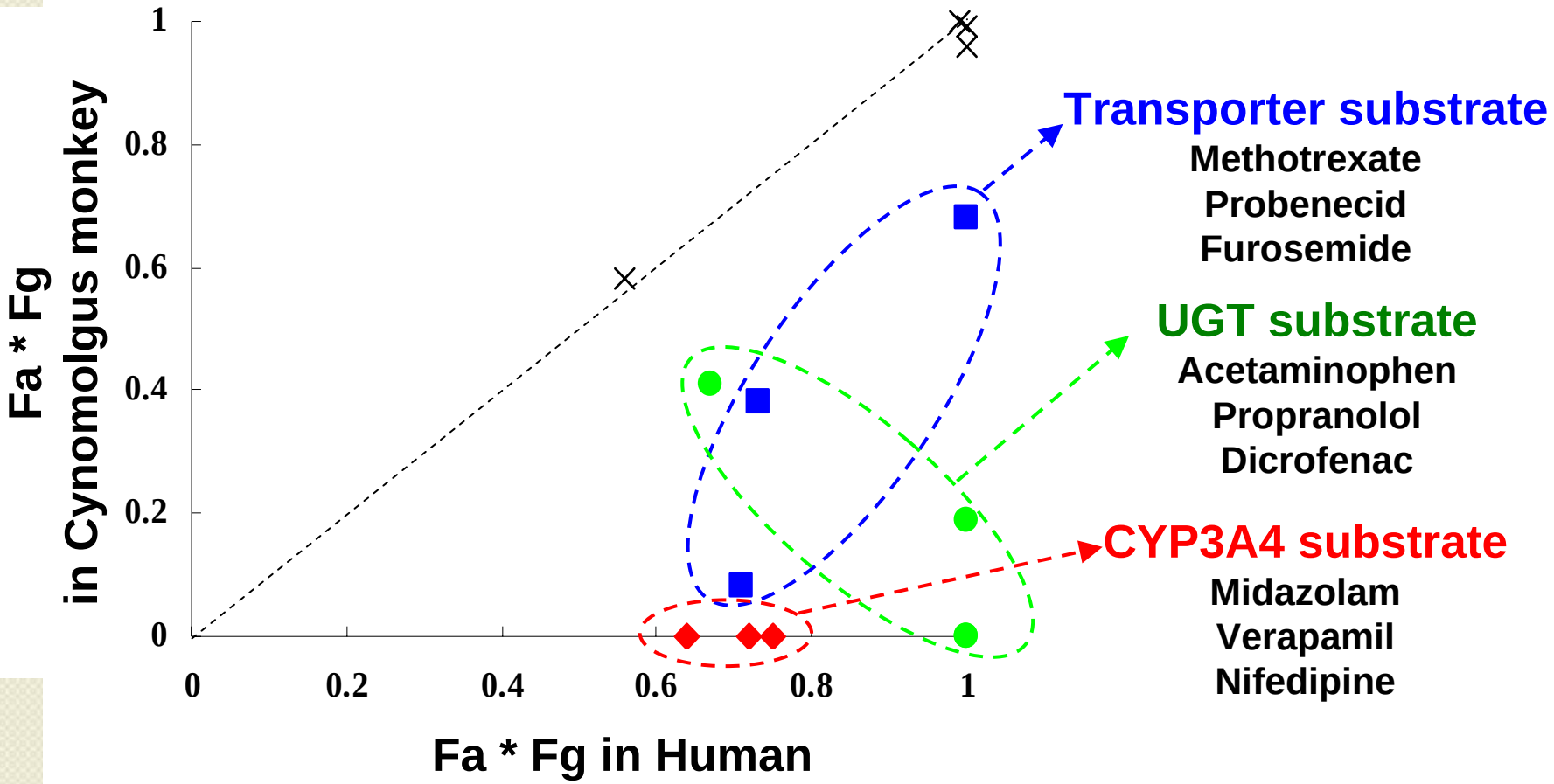


Species difference of BA between human and Cynomolgus monkey

compound	Dose		Oral Bioavailability (%)	
	Human	Monkey	Human	Monkey
Acetaminophen	20mg/kg	7.7 mg/kg	88	15.7
Atenolol	100mg	1 mg/kg	50	57
Furosemide	40mg	1 mg/kg	60	32
Methotrexate	80mg/m^2	0.5 mg/kg	70	8
Dicrofenac	50mg	0.5 mg/kg	54	29
Imipramine	200mg	0.5 mg/kg	42	<1
Propranolol	80mg	1 mg/kg	36	<1
Midazolam	10mg	0.5 mg/kg	44	<1
Verapamil	120mg	0.5 mg/kg	22	<1
Nifedipine	10mg	0.5 mg/kg	50	<1
Probenecid	500mg	0.5 mg/kg	100	66
Antipyrine	10mg/kg	0.5 mg/kg	100	85.1
Piroxicam	20mg	1 mg/kg	100	95
Naproxen	250mg	1 mg/kg	100	100

Takahashi M, *Washio T, Suzuki N, Igeta K, Yamashita S. J Pharm Sci.* ***98(11)****:4343-53. (2009)*

Species difference in Fa * Fg



Impact of intestinal enzymes and transporters

NEDO-Microdose project (2008 ~)

Project leader : Yuichi Sugiyama (The University of Tokyo)

PK analysis ⇔ **PET-Molecular Imaging**

Integration

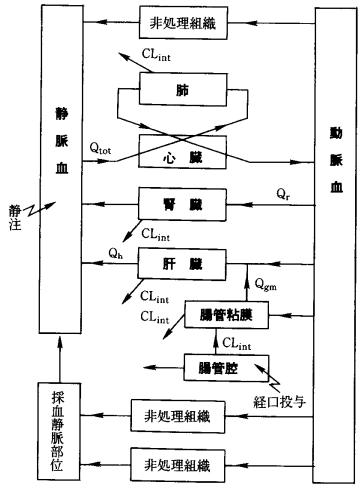
Microdose（MD）clinical study

Facilitation of Translation Research

At therapeutic dose

- Time-profile of drug conc. in plasma and tissue
- Therapeutic effect and side effect

Modeling & Simulation (in vitro＆in silico)



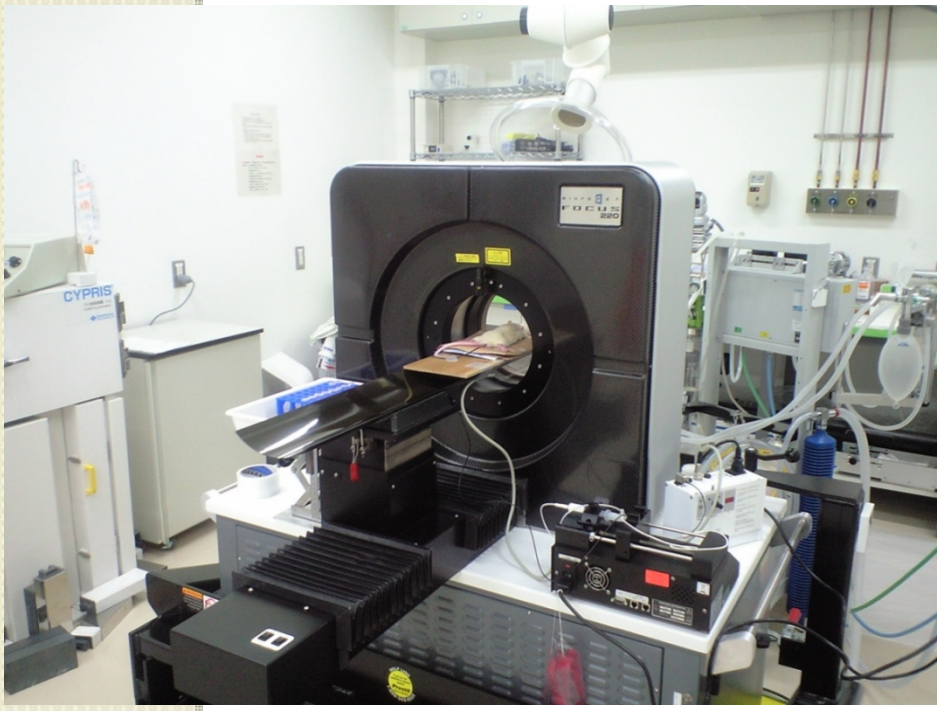
生理学的モデルの例

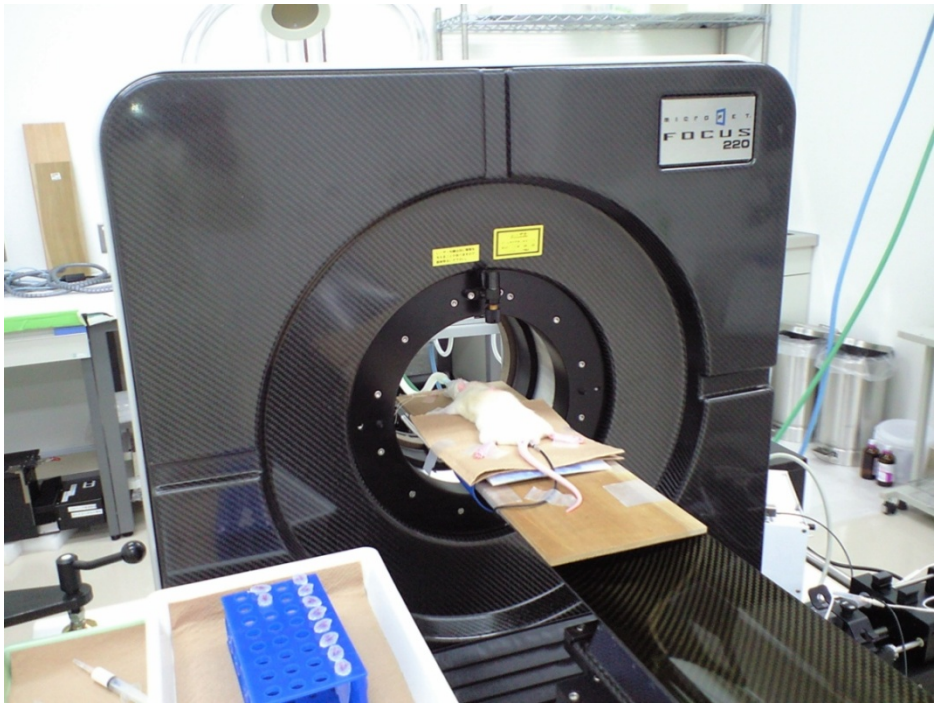
Goal

Increase success probability of drug development

Analysis of Oral Drug Absorption using PET molecular imaging

Probe compound : [^{18}F] FDG, [^{11}C]Telmisartan
Animal : Rat (0.5 mL/head)

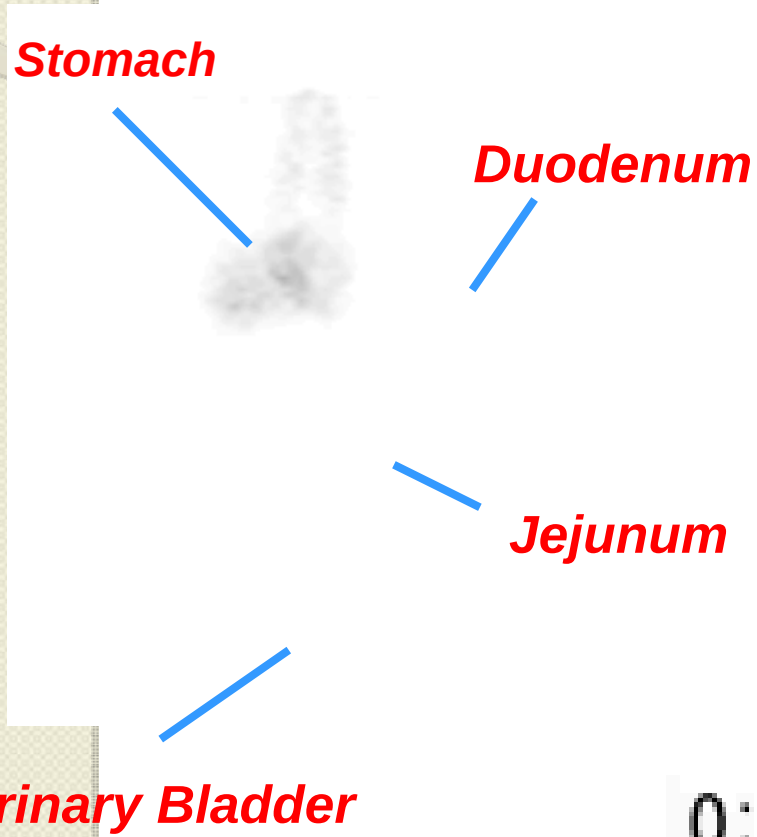




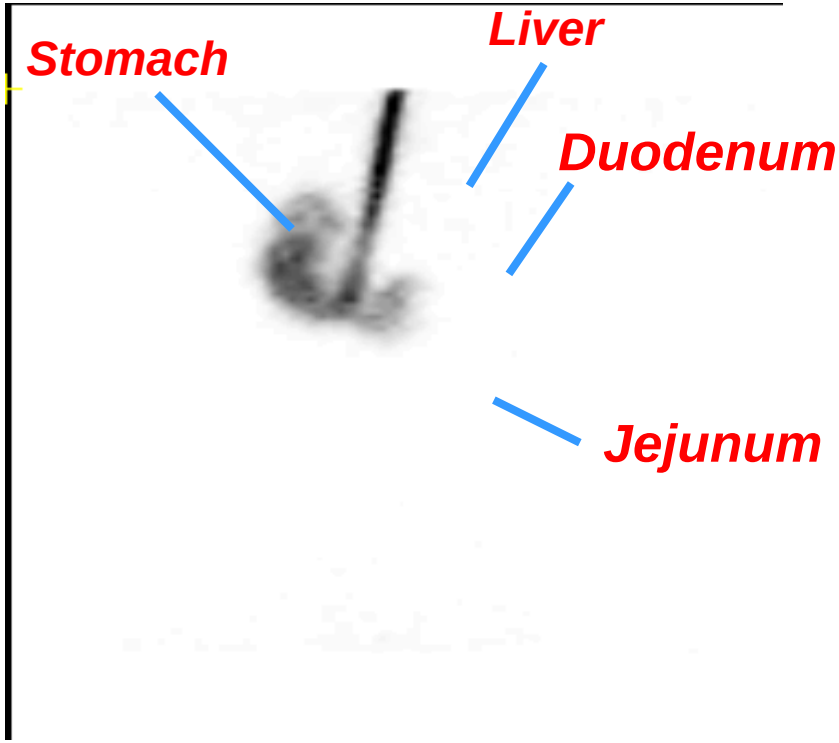
RIKEN Center for Molecular Imaging Science
Yamashita S, Takashima T, Kataoka M, Oh H, Sakuma S, Takahashi M, Suzuki N, Hayashinaka E, Wada Y, Cui Y, Watanabe Y.
J Nucl Med. ***52(2)****:249-56. (2011)*

Dynamic PET image of Abdominal Region after oral administration

[^{18}F]FDG



[^{11}C]Telmisartan



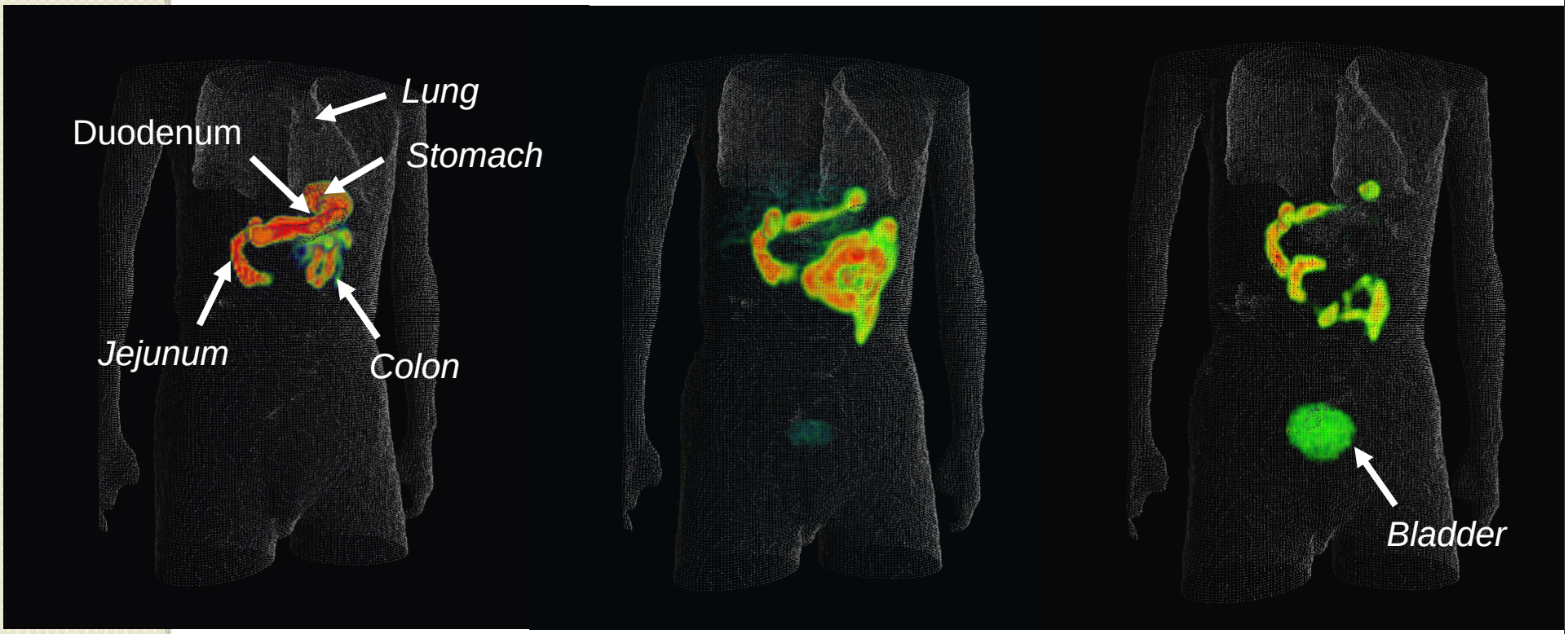
0:00:15

(h:mm:ss)

SUV: 0 — 20

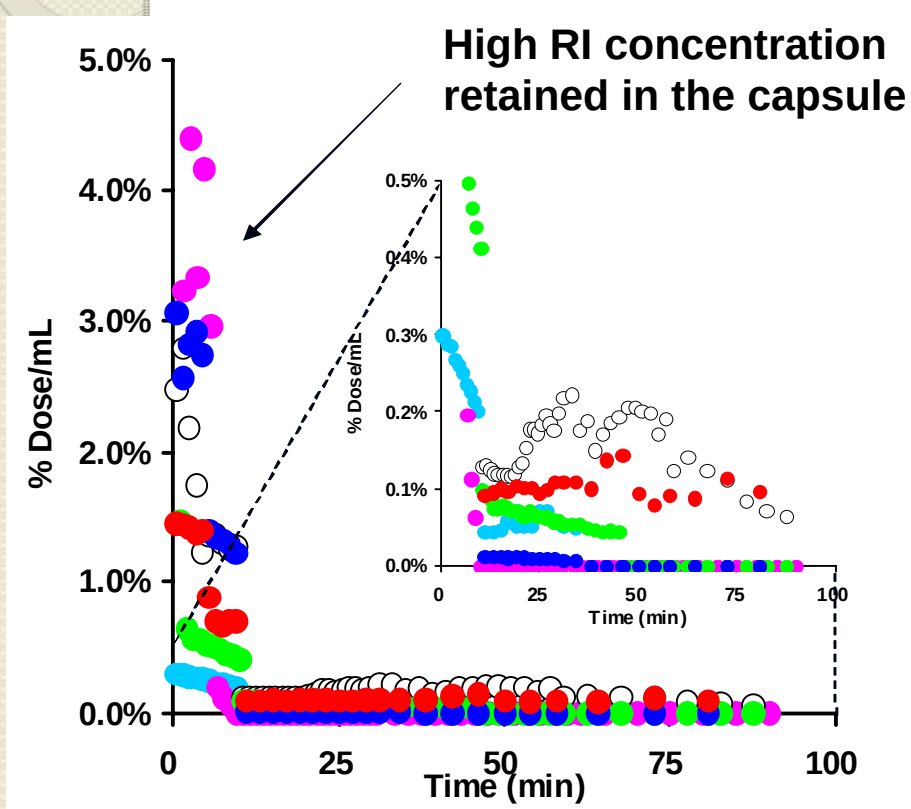
Human PET – PO study

"Whole body PET images" in human after oral administration of [^{18}F]FDG

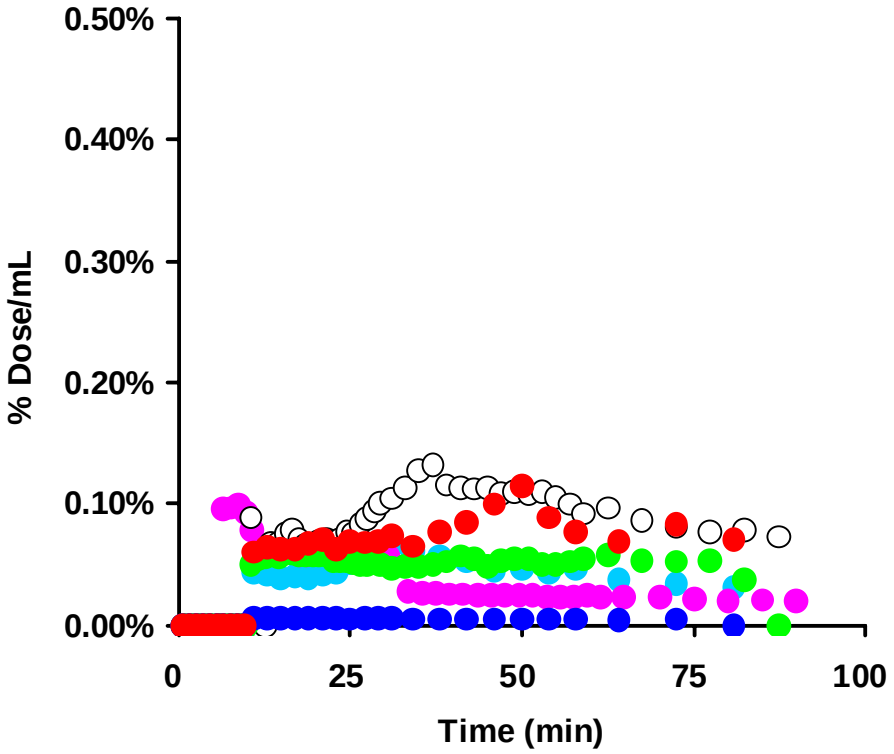


RI level (concentration: %ID/mL) at Stomach and Intestine after oral administration of [^{18}F]FDG

(stomach)



(intestine)



T Shingaki, T Takashima, Y Wada, M Tanaka, M Kataoka, A Ishii, Y Shigihara, Y Sugiyama, S Yamashita and Y Watanabe
Clin. Pharma.Therap. (2011) In press

Information required for development of oral drug products

Compound

Absorption potential

permeability

Challenging topics

- *screening of DDI*
- *screening id crystal form*
- *in silico simulation*

salt formation

Formulation design

Absorption profile

Challenging topics

- intestinal first-pass metabolism
- DDI
- food effect
- supersaturation
- lipid formulation

Product

Human PK profile

dose-linearity

Bioperformance of oral products

bioequivalence

BCS, BDDCS

IVIVC

Animal scale-up

Yamashita S*, Furubayashi T, Kataoka M, Sakane T, Sezaki H, **Tokuda H**.
Optimized conditions for prediction of intestinal drug permeability using Caco-2 cells.
Eur J Pharm Sci. 10(3):195-204 (2000)

Yamashita S*, Konishi K, Yamazaki Y, **Taki Y**, Sakane T, Sezaki H**, Furuyama Y**.
New and better protocols for a short-term Caco-2 cell culture system.
J Pharm Sci. 91(3):669-79. (2002)

Tamura S, Ibuki R, Tokunaga Y, Amidon GL, Sezaki H, Yamashita S.
The site-specific transport and metabolism of tacrolimus in rat small intestine.
J Pharmacol Exp Ther. 306(1):310-16. (2003)

Takano R, Sugano K, Higashida A, Hayashi Y, Machida M, Aso Y, Yamashita S.
Oral absorption of poorly water-soluble drugs: computer simulation of fraction absorbed in humans from a miniscale dissolution test. Pharm Res. 23(6):1144-56. (2006)

Masaoka Y, **Tanaka Y**, Kataoka M, Sakuma S, Yamashita S
Site of drug absorption after oral administration: Assessment of membrane permeability and luminal concentration of drugs in each segment. Eur J Pharm Sci. 29(3-4):240-50. (2006)

Yamashita S, **Tachiki H.**
Analysis of risk factors in human bioequivalence study that incur bioinequivalence of oral drug products. Mol Pharm. 6(1):48-59. (2009)

Yano K, Masaoka Y, Kataoka M, Sakuma S, Yamashita S.
Mechanisms of membrane transport of poorly soluble drugs: role of micelles in oral absorption processes. J Pharm Sci. 99(3):1336-45 (2010)

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Toshiyasu Sakane
Kazuko Miyagawa
Masako Yoshida
Yoko Taki
Yukako Yamazaki

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