DMPK

PKNCS, Kobe Pharma Research Institute, Japan

Development Germany

In Vitro Transporterデータに基づく薬物相互作用予測を目指した企業的研究

Prediction of drug interaction based on in vitro transporter data at pharmaceutical industry

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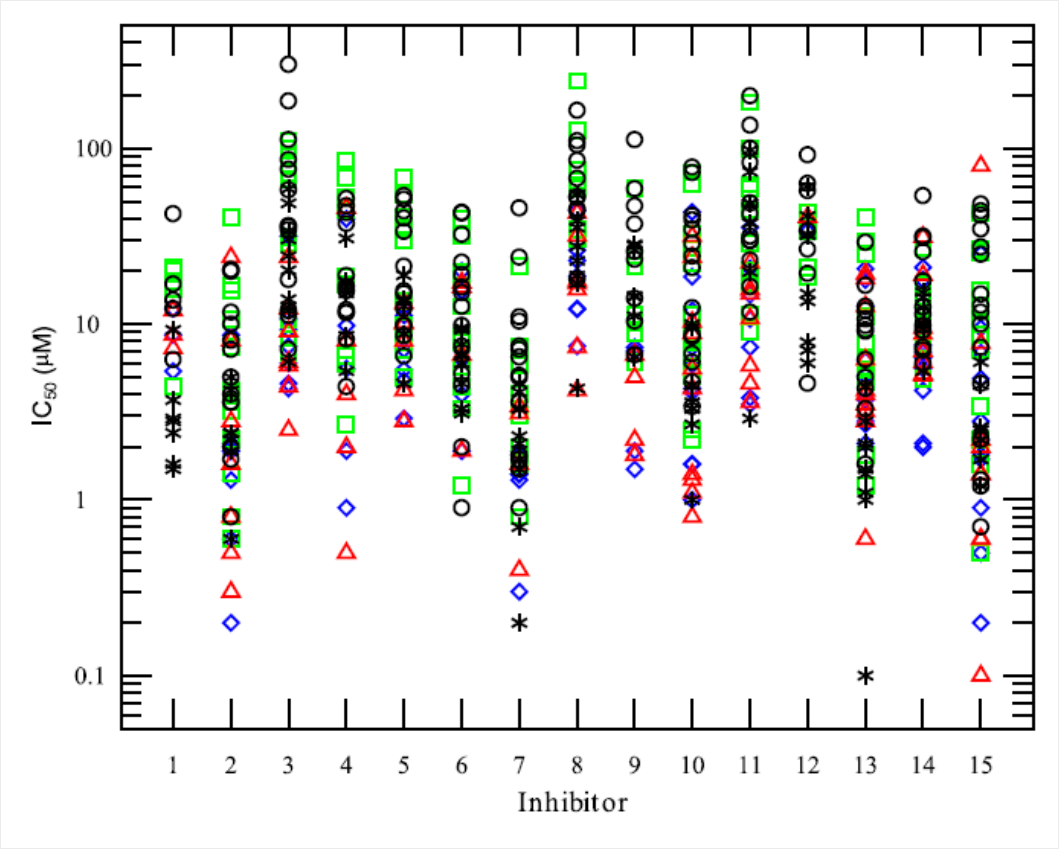
Inter-laboratory variability of in vitro P-gp IC_{50} values



Experimental systems used

- Caco2
- P-gp-LLC-PK1
- P-gp-MDCK
- P-gp-vesicle

Equations for IC_{50} calculations of data from the cell systems

- Effux ratio
- BtoA
- KP (AtoB)
- Net secretory flux

Inhibitors used

1 amiodarone, 2 carvedilol, 3 diltiazem, 4 felodipine, 5 isradipine, 6 mibefradil, 7 nicardipine, 8 nifedipine, 9 nitrendipine, 10 quinidine, 11 ranolazine, 12 sertraline, 13 telmisartan, 14 troglitazone, 15 verapamil

- Big variability (20-796 fold) in IC_{50} values was identified among 23 different laboratories, if difference in experimental conditions are not considered.

Bentz et al., 2013, Drug Metab Dispos. 41:1367-74.

Potential factors involved in variability of IC_{50} values

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Experimental system

- Cell system
  - P-gp-expressing LLC-PK1
  - P-gp-expressing MDCK
  - Caco2
- Cell free system
  - P-gp expressing vesicle

Experimental condition

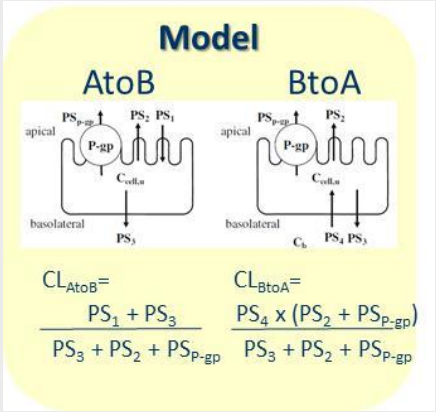
- In vitro probe substrate
- Substrate conc. (nd)
- pH gradient (nd)
- BSA supplementation
- Sink condition
- Buffer system (nd)

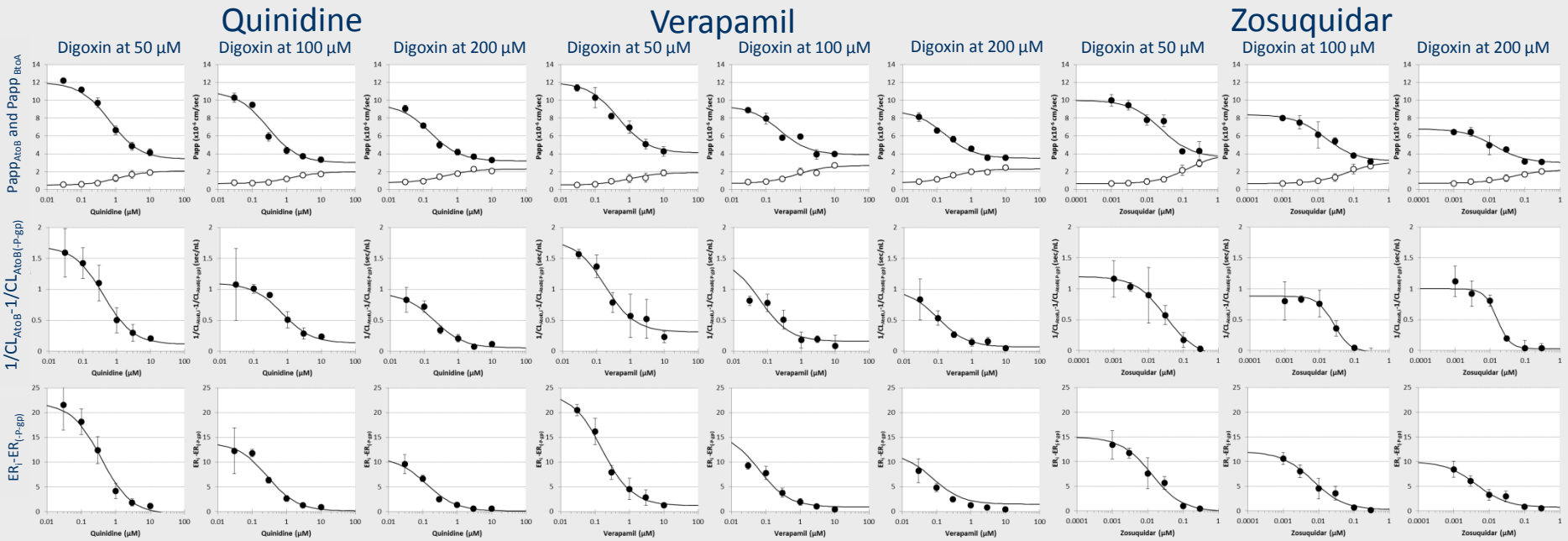
Experimental analysis

- Equations for IC_{50} calculation
  - Efflux ratio
  - BtoA
  - AtoB
  - BtoA-AtoB
  - etc

Experimental analysis
-Impact of IC_{50} calculation method (1)-

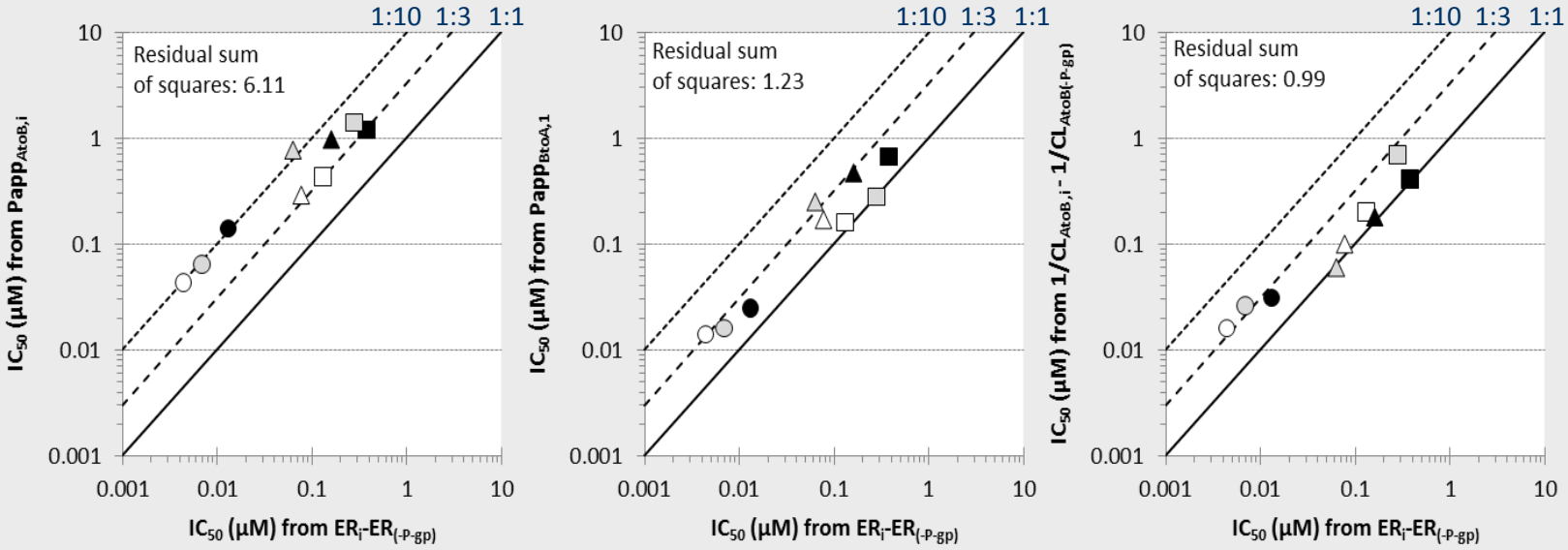
- IC_{50} calculation (4 equations)
  - *Empirical approach*
    - $CL_{AtoBi} = CL_{AtoB,i=\infty} + (CL_{AtoB,i=\infty} - CL_{AtoB,i=0}) \times \frac{1}{1+i/IC_{50}}$
    - $CL_{BtoAi} = CL_{BtoA,i=\infty} - (CL_{BtoA,i=0} - CL_{BtoA,i=\infty}) \times \frac{1}{1+i/IC_{50}}$
  - *Model based approach*
    - $ER_i - ER_{(-P\text{-}gp)} = \frac{PS_4 \times (PS_2 + PS_{P\text{-}gp,i})}{PS_1 \times PS_3} - \frac{PS_2 \times PS_4}{PS_1 \times PS_3} = \frac{PS_4}{PS_1 \times PS_3} \times PS_{P\text{-}gp,i=0} \times \frac{1}{1+i/IC_{50}}$
    - $1/CL_{AtoBi} - 1/CL_{AtoB(-P\text{-}gp)} = \frac{PS_3 + PS_2 + PS_{P\text{-}gp,i}}{PS_1 \times PS_3} - \frac{PS_2 \times PS_3}{PS_1 \times PS_3} = \frac{1}{PS_1 \times PS_3} \times PS_{P\text{-}gp,i=0} \times \frac{1}{1+i/IC_{50}}$





W.Kishimoto et al., JPS, accepted

Experimental analysis
-Impact of IC_{50} calculation method (2)-



○ zosuquidar	Closed: 50 μM
□ quinidine	Hatched: 100 μM
△ verapamil	Open: 200 μM

- AtoB vs ER: average 7-fold difference
- BtoA vs ER：average 2.3-fold difference
- $1/CL_{AtoB,i} - 1/CL_{AtoB(-P-gp)}$ vs ER: average 1.5 fold difference

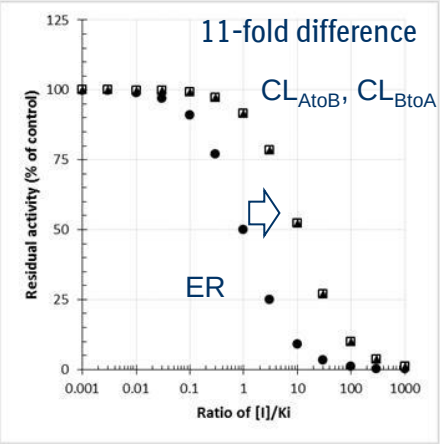
- **Even if same data set were used for IC_{50} calculation, IC_{50} values were different among methods used.**
- **There is no substantial difference in IC_{50} values between two different model based approaches**

W.Kishimoto et al., in preparation

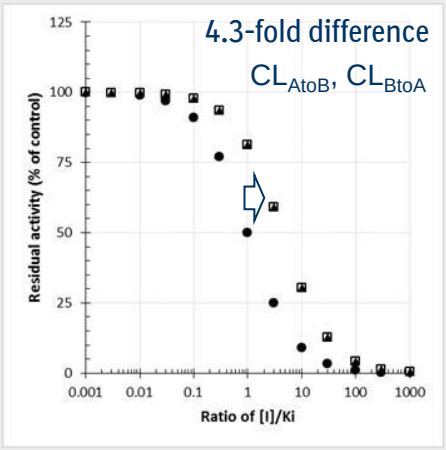
Experimental analysis
-Impact of IC_{50} calculation method (3)-

	Model based equation	P-gp active (low inhibitor conc.) (AtoB: $PS_2+PS_3 \ll PS_{Pgp}$) (BtoA: $PS_2+PS_{Pgp} \gg PS_3$)	P-gp inhibited (high inhibitor conc.) (AtoB: $PS_2+PS_3 \gg PS_{Pgp}$) (BtoA: $PS_2+PS_{Pgp} \ll PS_3$)
CL_{AtoB}	$\frac{PS_1+PS_3}{PS_3+PS_2+PS_{Pgp}}$	$\frac{PS_1+PS_3}{PS_{Pgp}}$	$\frac{PS_1+PS_3}{PS_3+PS_2}$
CL_{BtoA}	$\frac{PS_4 x (PS_2+PS_{Pgp})}{PS_3+PS_2+PS_{Pgp}}$	PS_4	$\frac{PS_4 x (PS_2+PS_{Pgp})}{PS_3+PS_2+PS_{Pgp}}$
ER_i-ER (-Pgp)	$\frac{PS_4 x PS_{Pgp}}{PS_1 x PS_3}$	$\frac{PS_4 x PS_{Pgp}}{PS_1 x PS_3}$	

$PS_1/PS_2/PS_3/PS_4/PS_{P\text{-}gp}$ =1/1/1/1/20



$PS_1/PS_2/PS_3/PS_4/PS_{P\text{-}gp}$ =1/1/5/1/20



- ✓ In case of CL_{AtoB} and CL_{BtoA}, there is a concentration range in which sensitivity against P-gp inhibitor is very low, resulting higher IC_{50} values compared to those obtained by model-based approaches.
- ✓ The fold difference between model and empirical approaches depends on probe substrate (ratio of PS_1-PS_{Pgp}).

Summary

- It is recently known that there is relatively big data variability in experimental data for efflux transporters such as P-gp and BCRP

- Experimental analysis
  - There is difference in IC_{50} between empirical and model based approaches which would contribute at least partly inter-laboratory difference
  - Model-based approach such as our new approach $[1/CL_{AtoB,i} - 1/CL_{AtoB(-P\text{-}gp)}]$ and ERi-ER(-P-gp) would be suitable for IC_{50} estimation from transcellular transport study, because the IC_{50} values from model-based approaches directly reflect the P-gp function.