2014 JSSX Award for Young Industrial Scientists

Development of predictive method of unbound brain concentration of central nervous system drugs for drug discovery

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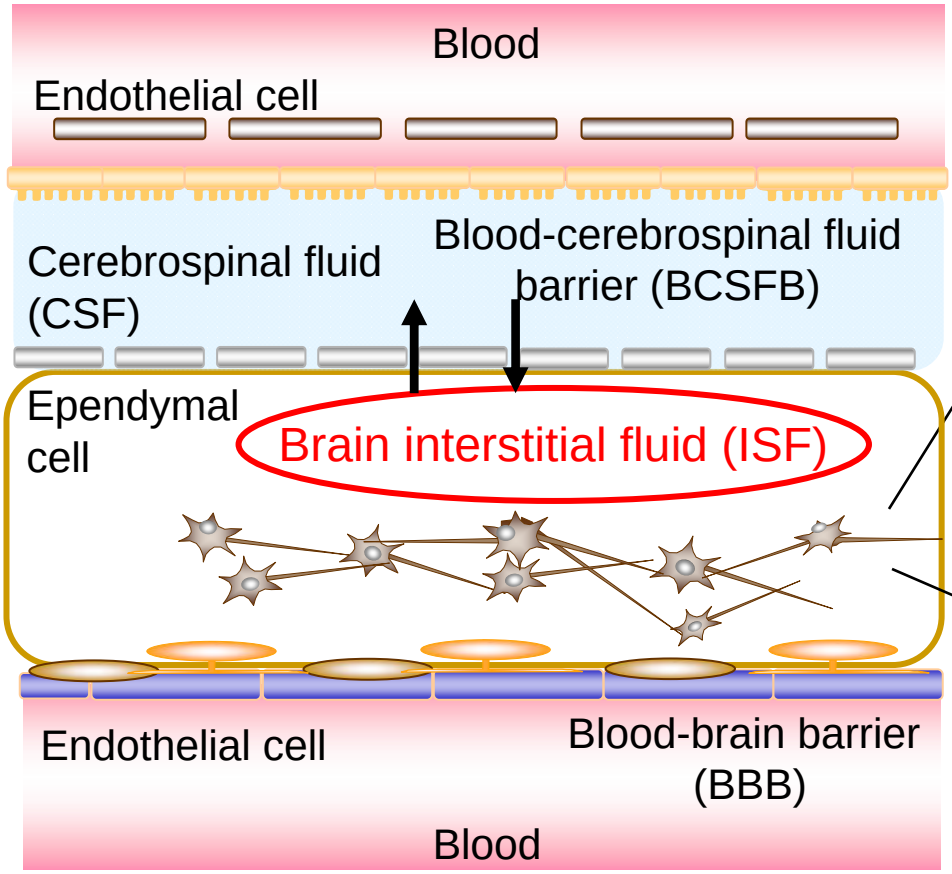
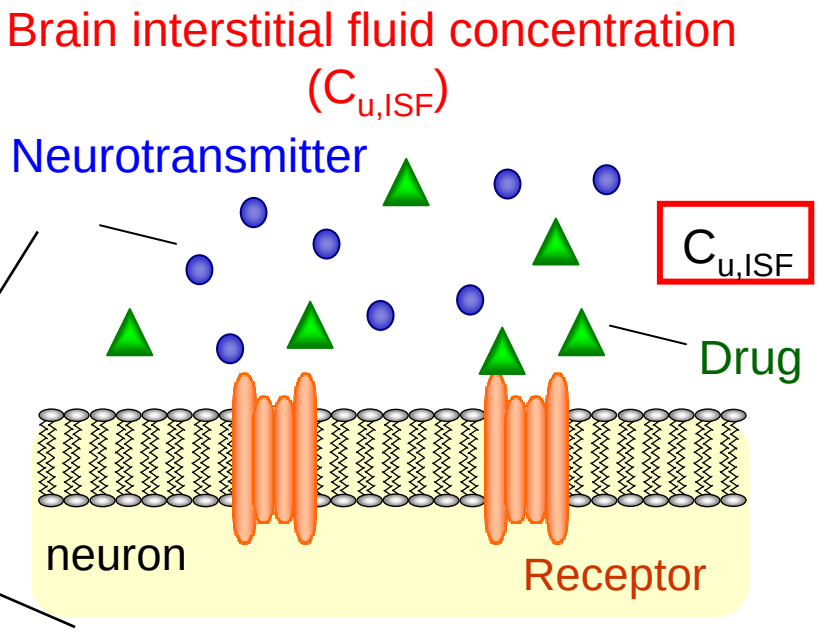
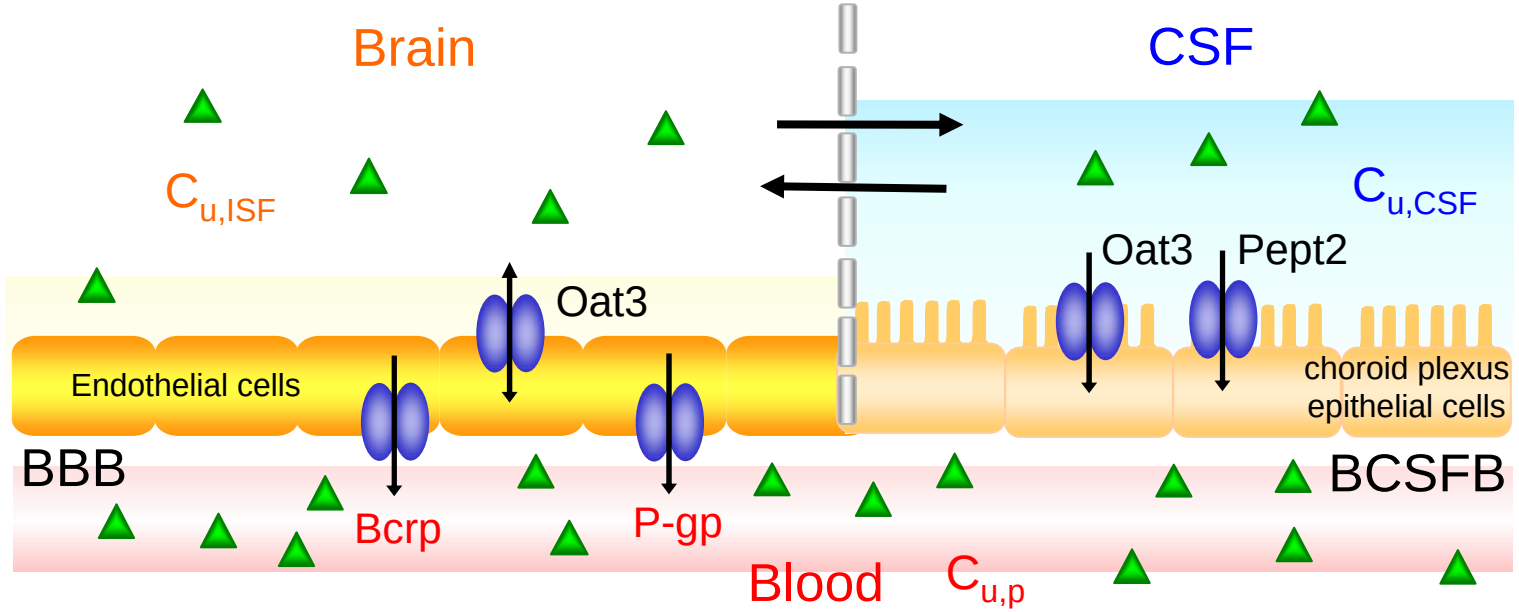
CNS disposition of drugs with regard to their CNS effect





Inhibition/activation of the target protein is determined by the unbound concentration of drugs in ISF ($C_{u,ISF}$).

Difficulty to determine and predict $C_{u,ISF}$



$C_{u,p}$: Unbound drug concentration in the blood
$C_{u,CSF}$: drug concentration in the CSF

Method to determine $C_{u,ISF}$

Microdialysis ⇒ It is difficult to apply the technique in the drug discovery stage because of high resource requirements and low throughput.

Indicators of $C_{u,ISF}$

$C_{u,p}$ ⇒It is not necessarily a surrogate of $C_{u,ISF}$ because of active efflux by xenobiotic transporters.

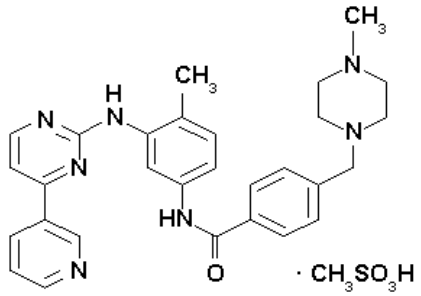
Development of prediction method of unbound brain concentration of central nervous system drugs for drug discovery.

I. Quantitative analysis of the drug efflux transport by P–gp and Bcrp at the BBB.

II. Quantitative analysis of the relationship of unbound concentration of drugs in the brain interstitial space and cerebrospinal fluid.

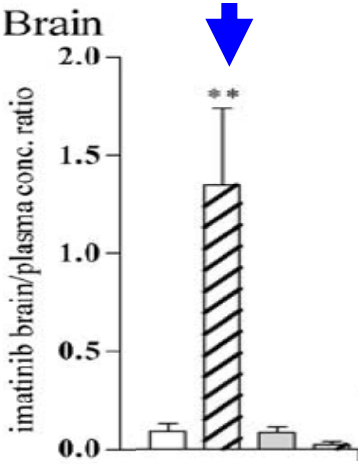
Drug efflux mechanism by P-gp and Bcrp at the BBB

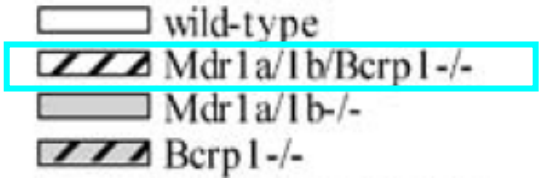
Imatinib



A common substrate of P-gp and Bcrp

Brain distribution of imatinib





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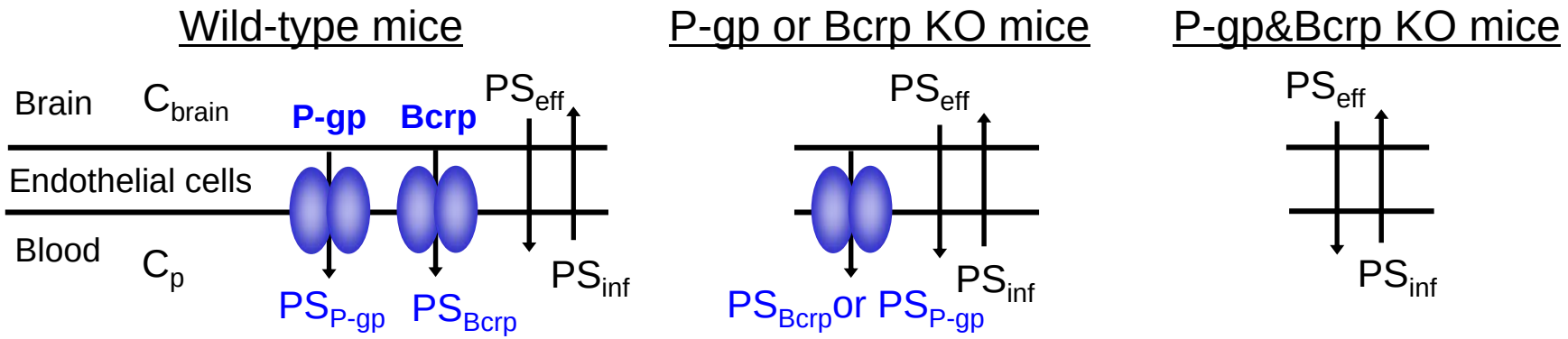
Oostendorp RL, et al. Invest New Drugs 27:31-40, 2009

Imatinib and some drugs exhibited a considerable increase in brain concentration in Mdr1a/1b/Bcrp$^{-/-}$ mice.

- Flavopiridol, Imatinib, and Prazosin; Zhou L et al., *Drug Metab Dispos.*37:946-55*. 2009*
- Dasatinib; Chen, Y et al., *J Pharmacol Exp Ther*.330:956-63. 2009
- Lapatinib; Polli JW et al., *Drug Metab Dispos.* 37:439-442. 2009
- Topotecan; de Vries NA et al., *Clin Cancer Res.* 13:6440-9. 2007

Kinetic analysis of drug efflux mediated by P–gp and Bcrp



C_{brain}/C_p ($K_{p,brain}$) of each KO mice to wild-type mice

P-gp KO/WT

$$= 1 + \frac{PS_{P\text{-}gp}}{PS_{eff} + PS_{Bcrp}}$$

Bcrp KO/WT

$$= 1 + \frac{PS_{Bcrp}}{PS_{eff} + PS_{P\text{-}gp}}$$

P-gp&Bcrp KO/WT

$$= 1 + \frac{PS_{P\text{-}gp} + PS_{Bcrp}}{PS_{eff}}$$

Ex) PS_{eff}=1
$PS_{P\text{-}gp}$=10
PS_{Bcrp}=10

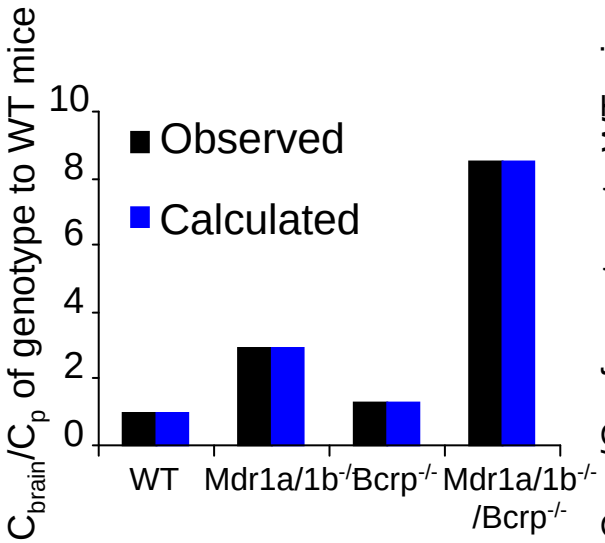
	P-gp KO /WT	Bcrp KO /WT	P-gp/Bcrp KO /WT
C_{brain}/C_p	**1.9**	**1.9**	**21**

Hypothesis：Contribution of P-gp and Bcrp to the net efflux at the BBB explains the apparent synergistic effect.

Kusuhara H. and Sugiyama Y Drug Metab Pharmacokinet. 24:37-52. 2009

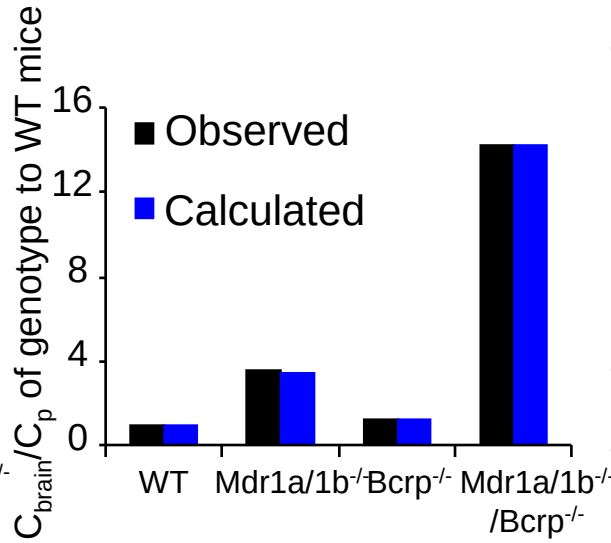
PS products of P–gp and Bcrp–mediated efflux transport of the common substrates

Erlotinib



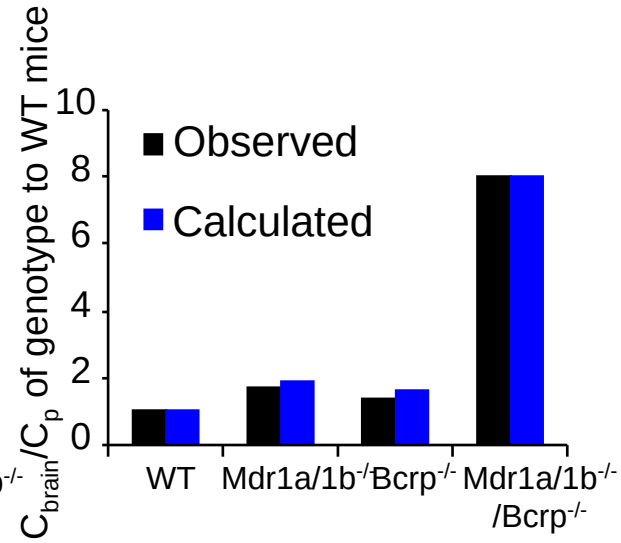
PS_{Bcrp}	$PS_{P\text{-}gp}$
1.89 ± 0.01	5.63 ± 0.01

Flavopiridol



PS_{Bcrp}	$PS_{P\text{-}gp}$
3.07 ± 0.02	10.1 ± 0.0

Mitoxantrone



PS_{Bcrp}	$PS_{P\text{-}gp}$
3.14 ± 0.55	3.86 ± 0.56

A kinetic consideration could reasonably explained the increase in the brain concentrations in Mdr1a/1b$^{-/-}$/Bcrp$^{-/-}$ mice.

Kodaira et al., *J Pharmacol Exp Ther*. 2010

Application of the kinetic approach to the previously published data

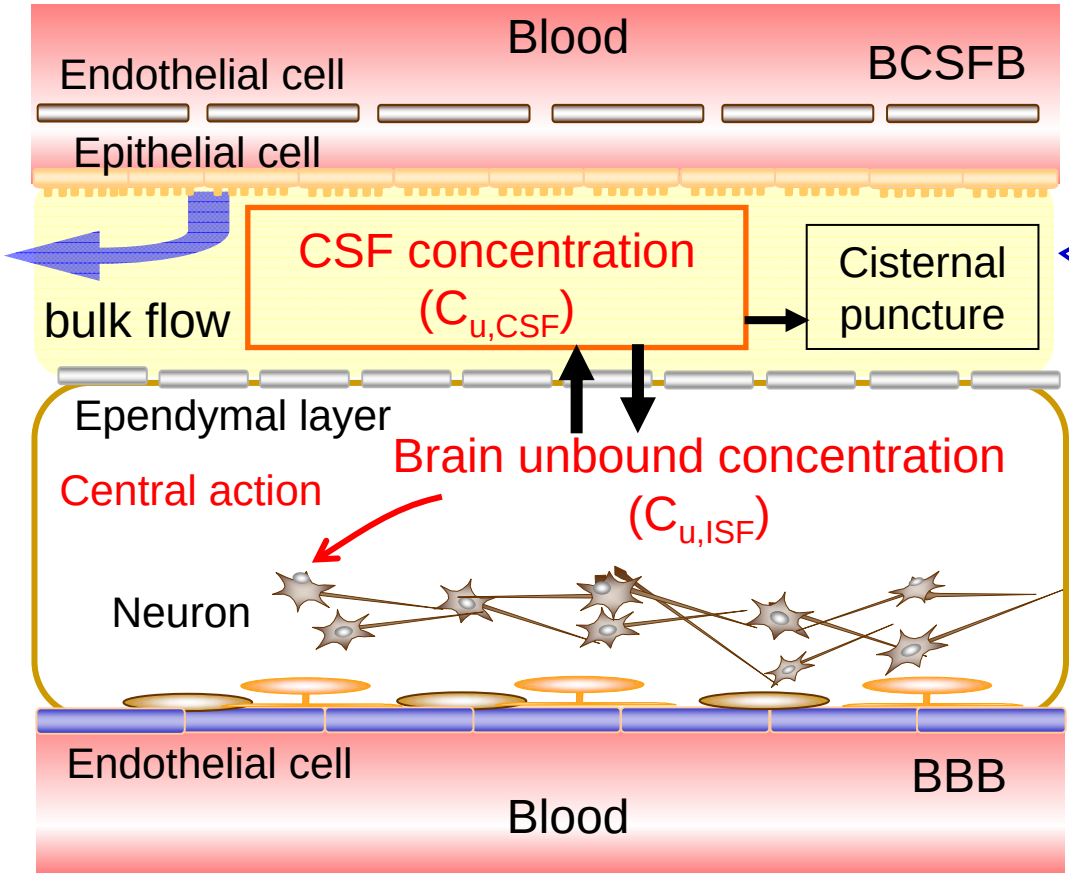
Compound	PS_{Bcrp}	$PS_{P\text{-}gp}$		$K_{p,brain}$ in each KO mice to WT mice		
				P-gp KO	Bcrp KO	P-gp/Bcrp KO
Flavopiridol[a]	2.83 ± 0.71	3.50 ± 0.71	Obs. / Cal.	1.7 / 1.9	1.3 / 1.6	7.4 / 7.3
	2.73 ± 0.69	3.15 ± 0.70		1.6 / 1.8	1.3 / 1.7	6.9 / 6.9
Dasatinib[b]	1	7	Obs. / Cal.	4 / 5	1 / 1	9 / 9
Imatinib[a]	5.31 ± 0.42	21.9 ± 0.5	Obs. / Cal.	4.5 / 4.5	0.94 / 1.2	28 / 28
	13.2 ± 1.3	49.4 ± 1.3		4.5 / 4.5	0.86 / 1.3	64 / 64
Imatinib[c]	4.4 ± 1.1	7.9 ± 1.1	Obs. / Cal.	2.3 / 2.5	1.0 / 1.5	13 / 13
	5.8 ± 3.2	5.8 ± 3.2		1.0 / 1.9	1.0 / 1.9	13 / 13
Lapatinib[d]	12.2 ± 0.5	26.8 ± 0.5	Obs. / Cal.	3.0 / 3.0	1.3 / 1.4	40 / 40
	9.55 ± 0.76	32.0 ± 0.8		4.0 / 4.0	1.0 / 1.3	43 / 43
Prazosin[a]	1.72 ± 0.16	2.51 ± 0.17	Obs. / Cal.	1.9 / 1.9	1.4 / 1.5	5.2 / 5.2
	1.63 ± 0.53	2.52 ± 0.54		1.8 / 2.0	1.1 / 1.5	5.2 / 5.2

a) Zhou L et al. Drug Metab Dispos. 37:946-955. 2009, b) Chen Y et al. J Pharmacol Exp Ther. 330:956-963. 2009, c) Oostendorp RL et al. Invest New Drugs. 27:31-40. 2009, d) Polli JW et al. Drug Metab Dispos. 37:439-442. 2009

The synergistic effect of P-gp and Bcrp can be explained by their contribution to the net efflux at the BBB.

Kodaira et al., *J Pharmacol Exp Ther*. 2010

$C_{u,CSF}$ as a surrogate of $C_{u,ISF}$

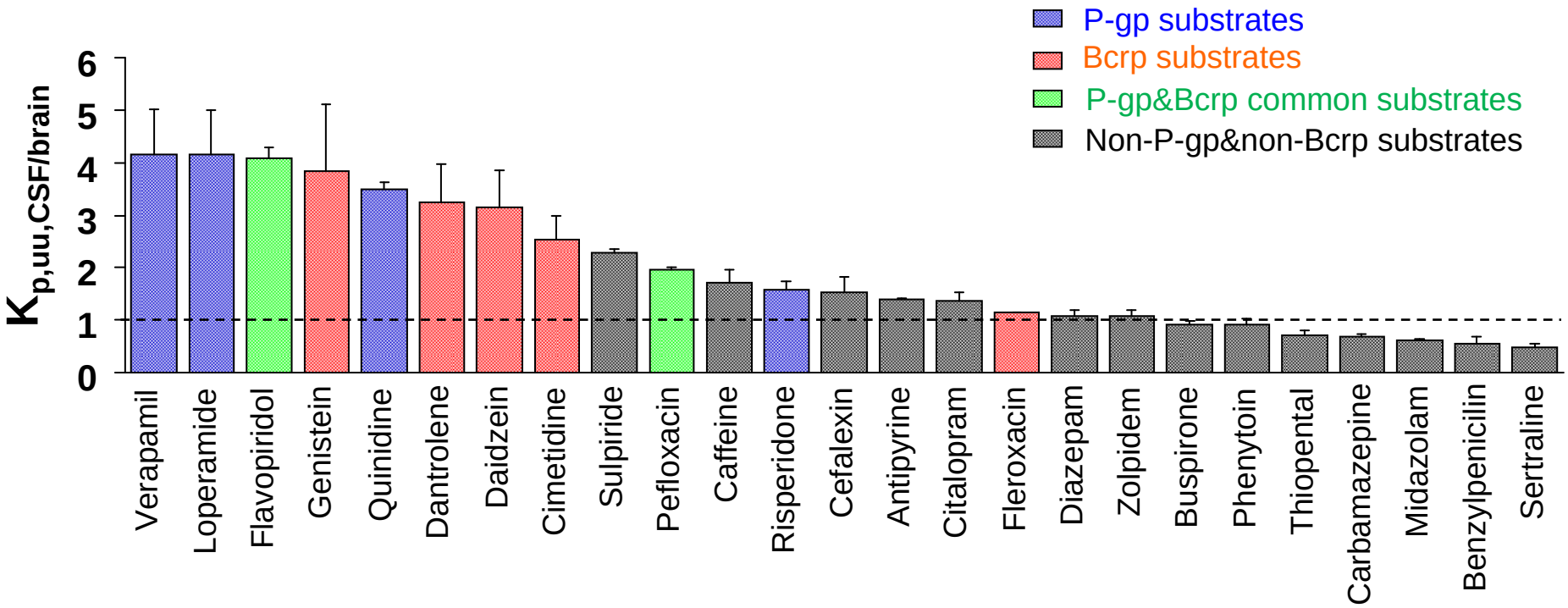


・Protein concentrations in CSF is extremely low.
・Ependyma allows the free exchange of drugs.

CSF has been used as a surrogate of $C_{u,ISF}$.

Problems:
・Some drugs exhibit a difference between $C_{u,CSF}$ and $C_{u,brain}$.
・Prediction method of such a concentration difference has not been established yet.

CSF–to–brain unbound concentration ratio of 25 drugs



Rats were given drugs by intravenous infusion.
Unbound fraction in the brain ($f_{u,brain}$) was determined by brain slice method.
Unbound concentration in the brain ($C_{u,ISF}$) is the product of C_{brain} and $f_{u,brain}$.

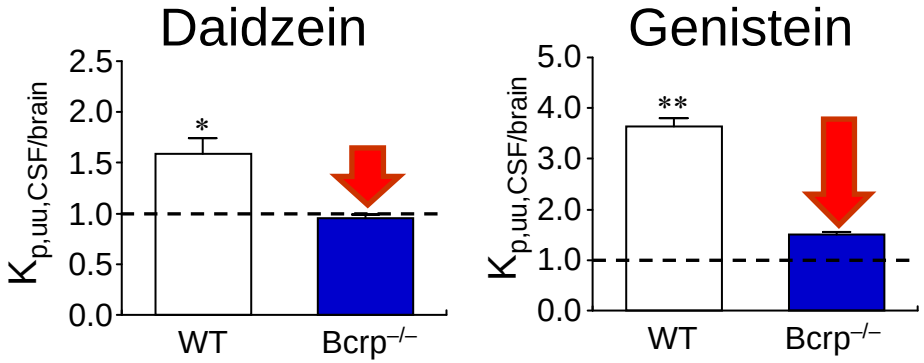
N=3-5, Mean±SE

P-gp and/or Bcrp substrates showed higher $C_{u,CSF}$ than $C_{u,ISF}$.

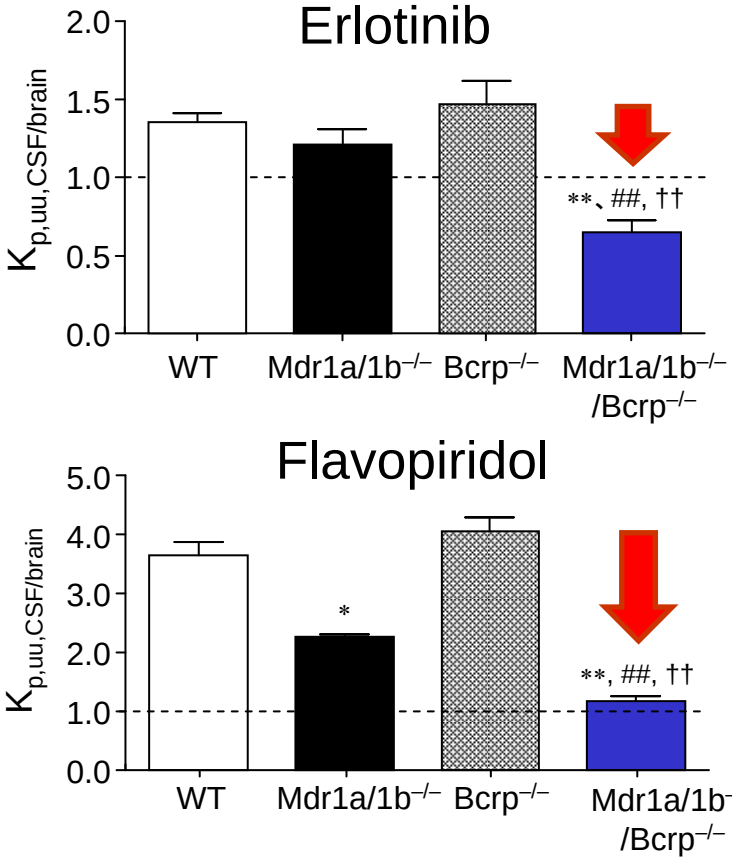
Kodaira et al., *J Pharmacol Exp Ther*. 2011

Effect of efflux transport of P-gp and/or Bcrp on difference between $C_{u,ISF}$ and $C_{u,CSF}$

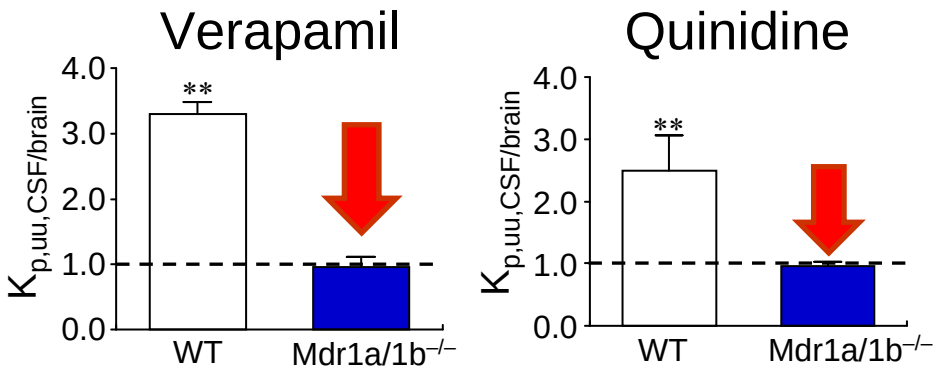
Bcrp substrate



P-gp&Bcrp substrate



P–gp substrate



*P < 0.05 and **P < 0.01, WT mice *vs* Mdr1a/1b$^{-/-}$ or Bcrp$^{-/-}$, n=3-4, mean±SE

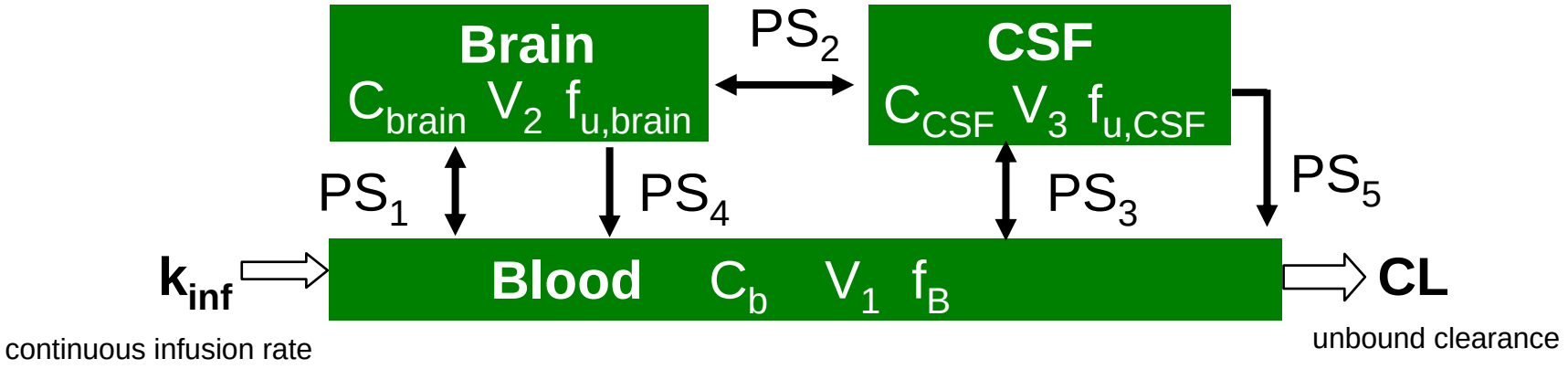
**P < 0.01, WT mice vs Mdr1a/1b$^{-/-}$, or Mdr1a/1b$^{-/-}$/Bcrp$^{-/-}$ mice; ## P < 0.01, Mdr1a/1b$^{-/-}$ vs Mdr1a/1b$^{-/-}$/Bcrp$^{-/-}$ mice; †† P < 0.01, Bcrp$^{-/-}$ vs Mdr1a/1b$^{-/-}$/Bcrp$^{-/-}$ mice 、N=3, Mean±SE

The difference between $C_{u,ISF}$ and $C_{u,CSF}$ is attributed to P-gp- and Bcrp-mediated efflux at the BBB.

Kodaira et al., *J Pharmacol Exp Ther*. 2011

Kinetic analysis to predict difference between $C_{u,CSF}$ and $C_{u,ISF}$

Three–compartment model



Concentration(C): C_b, C_{brain}, CSF: blood, brain, and CSF concentration

Distribution volume(V): V_1, V_2, V_3: distribution volume in the blood, brain, and CSF for unbound drug

Unbound fraction(f): f_B, $f_{u,brain}$, $f_{u,CSF}$: unbound fraction in blood, plasma, brain, and CSF

Clearance(PS): PS_1:passive permeability CL at the BBB, PS_2: diffusion between brain and CSF, PS_3:passive permeability CLat the BCSFB, PS_4: active efflux CL from brain to blood, PS_5:bulk flow of CSF

Mass balance equations

Blood: $$V_1 \cdot \frac{df_b \cdot C_B}{dt} = -(PS_1 + PS_3) \cdot f_b \cdot C_B - CL \cdot f_b \cdot C_B + k_{inf} + (PS_1 + PS_4) \cdot f_{u,brain} \cdot C_{brain} + (PS_3 + PS_5) \cdot f_{u,CSF} \cdot C_{CSF}$$

Brain: $$V_2 \cdot \frac{df_{u,brain} \cdot C_{brain}}{dt} = PS_1 \cdot f_b \cdot C_B - (PS_1 + PS_2 + PS_4) \cdot f_{u,brain} \cdot C_{brain} + PS_2 \cdot f_{u,CSF} \cdot C_{CSF}$$

CSF: $$V_3 \cdot \frac{df_{u,CSF} \cdot C_{CSF}}{dt} = PS_3 \cdot f_b \cdot C_B + PS_2 \cdot f_{u,brain} \cdot C_{brain} - (PS_2 + PS_3 + PS_5) \cdot f_{u,CSF} \cdot C_{CSF}$$

Kinetic analysis to predict difference between $C_{u,CSF}$ and $C_{u,ISF}$

Under steady–state conditions, $K_{p,uu,brain}$ and $K_{p,uu,CSF}$ are given by:

$$K_{p,uu,brain} = \frac{1 + \frac{PS_5}{PS_3} + \frac{PS_2}{PS_1} + \frac{PS_2}{PS_3}}{Z} \qquad K_{p,uu,CSF} = \frac{1 + \frac{PS_4}{PS_1} + \frac{PS_2}{PS_1} + \frac{PS_2}{PS_3}}{Z}$$

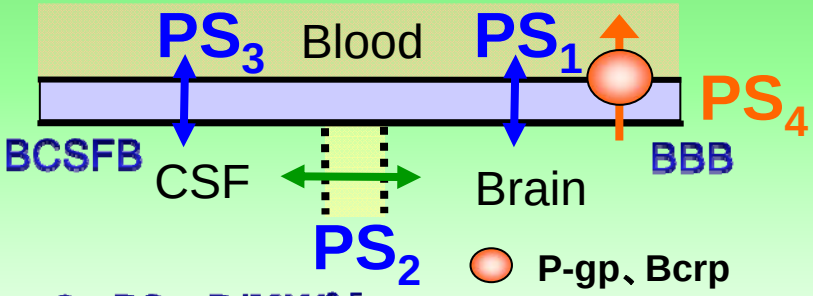
$$Z = 1 + \frac{PS_2 + PS_4}{PS_1} + \frac{PS_2 + PS_5}{PS_3} + \frac{PS_2 PS_4 + PS_5 (PS_2 + PS_4)}{PS_1 PS_3}$$

Assumption

In vivo

1. $PS_1 = A \cdot PS_3$

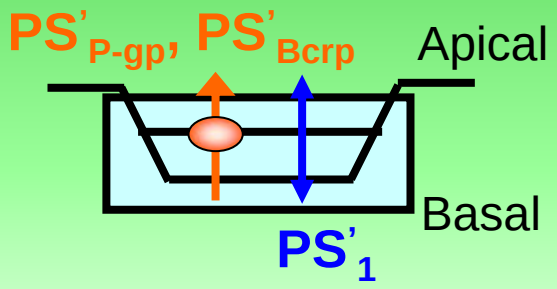
PS_3 is proportional to PS_1.



2. $PS_2 = D/MW^{0.5}$

The drug exchange between brain and CSF is diffusion.

In vitro transport activity



3. $PS_4/PS_1 = B \cdot PS'_{P\text{-}gp}/PS'_1 + C \cdot PS'_{Bcrp}/PS'_1$

In vivo $PS_{P\text{-}gp}$ or PS_{Bcrp} is proportional to in vitro that.

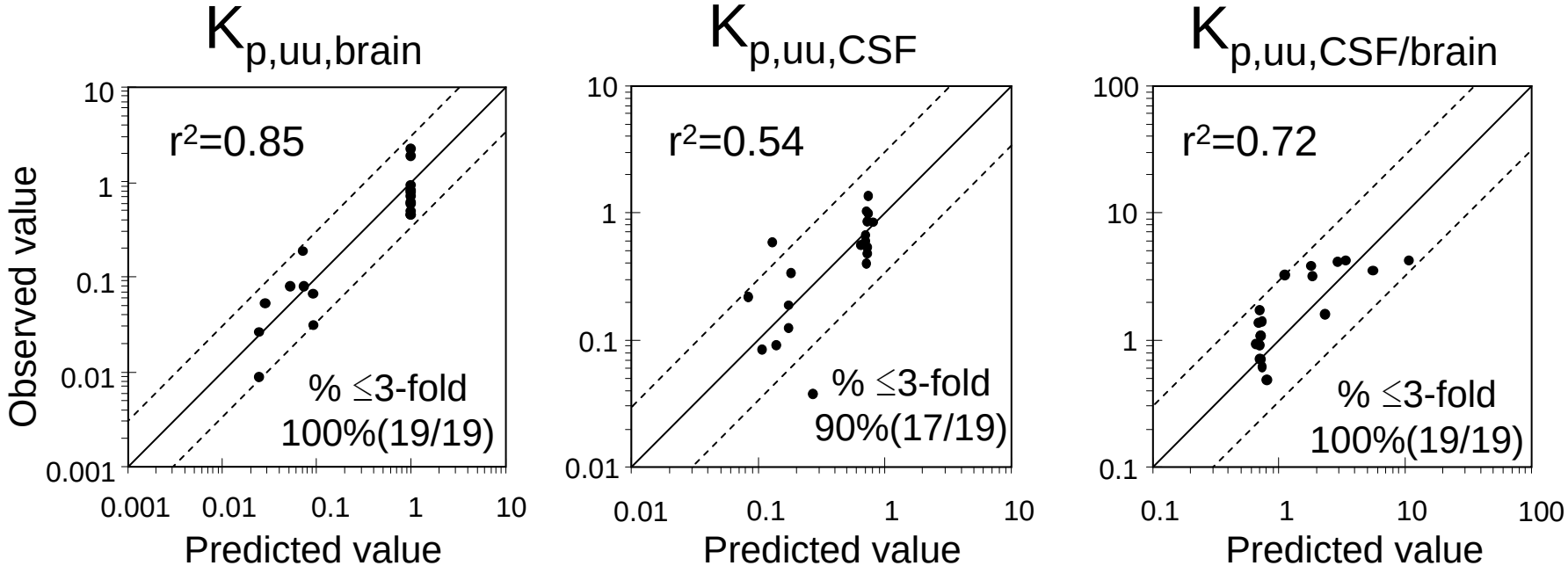
A, B, C, D: fitted parameters

Comparison of predicted and observed unbound concentration ratio ($K_{p,uu,brain}$, $K_{p,uu,CSF}$, $K_{p,uu,CSF/brain}$)

- **In vitro transporter activity**
- **in silico BBB permeability**

A non-linear least squares method

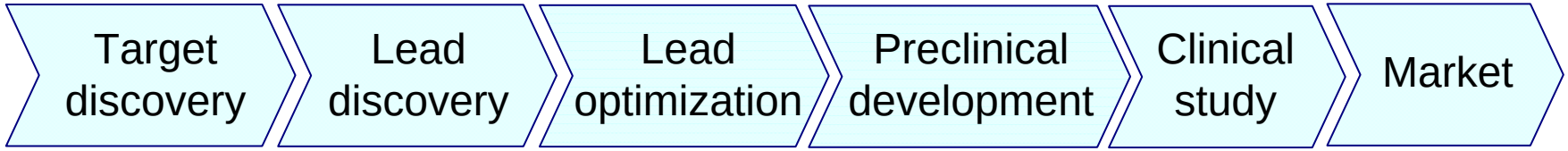
In vivo $K_{p,uu,brain}$, $K_{p,uu,CSF}$



Kodaira et al., *Drug Metab Dispos. 2014*

The developed model can reasonably describe the $K_{p,uu,CSF/brain}$ of drugs.

Drug discovery and development flow

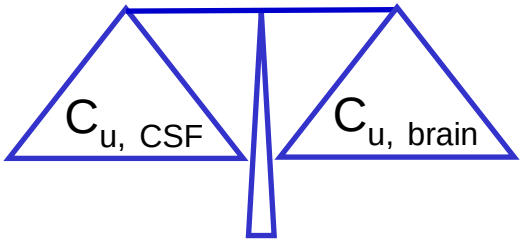


New Chemical Entities for treatment of CNS

in vitro / in silico data

- in vitro transporter activity (P-gp/Bcrp)
- in silico BBB permeability

⇒ $K_{p,uu,\ CSF/brain}$



The developed model will suggest the reliability of the predicted $C_{u,ISF}$ of CNS drugs using $C_{u,CSF}$ at early stage of drug discovery

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PKPD seminar

(Prof. Sugiyama preside over)

All researchers