

Restricting brain penetration of drugs by targeting drug transporters

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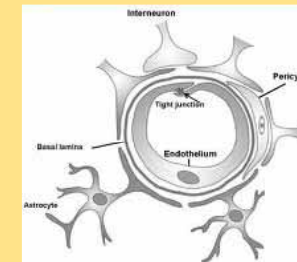
RSC Drug Transporters Symposium:
Target or Avoid?

13th November 2014

introduction



- Neusentis is a Pfizer research unit focussed on pain and sensory disorders
- located in (near) Cambridge, UK
- largely peripheral drug targets for treatment of pain and sensory disorders
- interest in understanding brain penetration



outline

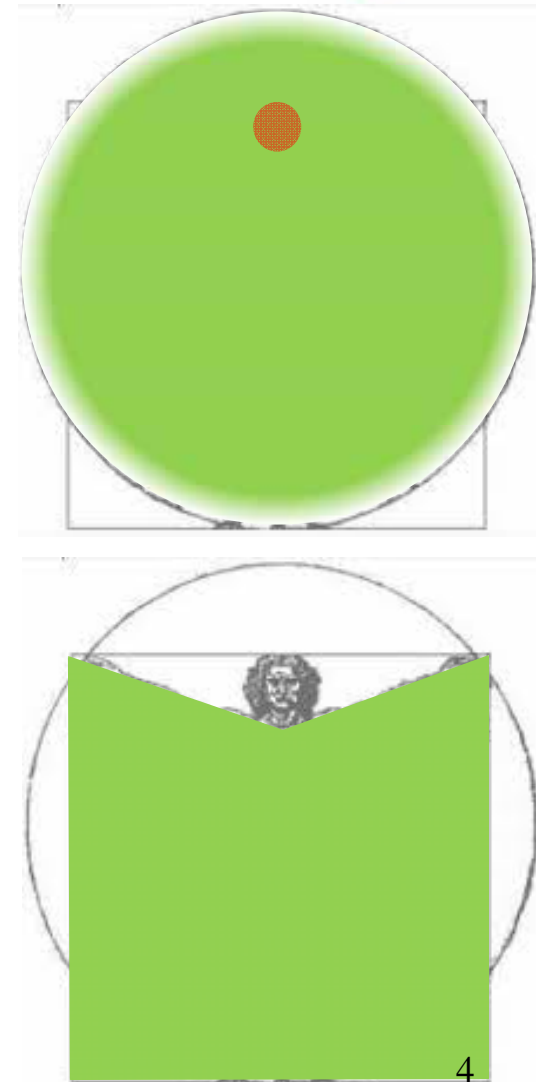
- ❑ Potential advantages of limiting brain penetration (CNS restriction)
- ❑ Role of active efflux transporters in CNS restriction of drugs
- ❑ Laboratory methods used to study efflux transporters and CNS restriction
- ❑ Risks with targeting active efflux to achieve CNS restriction
 - ❑ absorption following oral administration
 - ❑ drug-drug interactions
 - ❑ variation in transporter activity in patient populations
- ❑ Conclusions

drug safety & tolerability

- ❑ some drug discovery approaches target peripheral (non-CNS) receptors
- ❑ unwanted side-effects may arise due to interaction with receptors in the CNS
- ❑ minimizing exposure in CNS has the benefit of reducing risk of side-effects originating in the CNS
- ❑ the blood-brain barrier (BBB) restricts access of some xenobiotics to the CNS
- ❑ can we exploit the BBB to minimize risk of CNS side-effects and improve safety and tolerability?

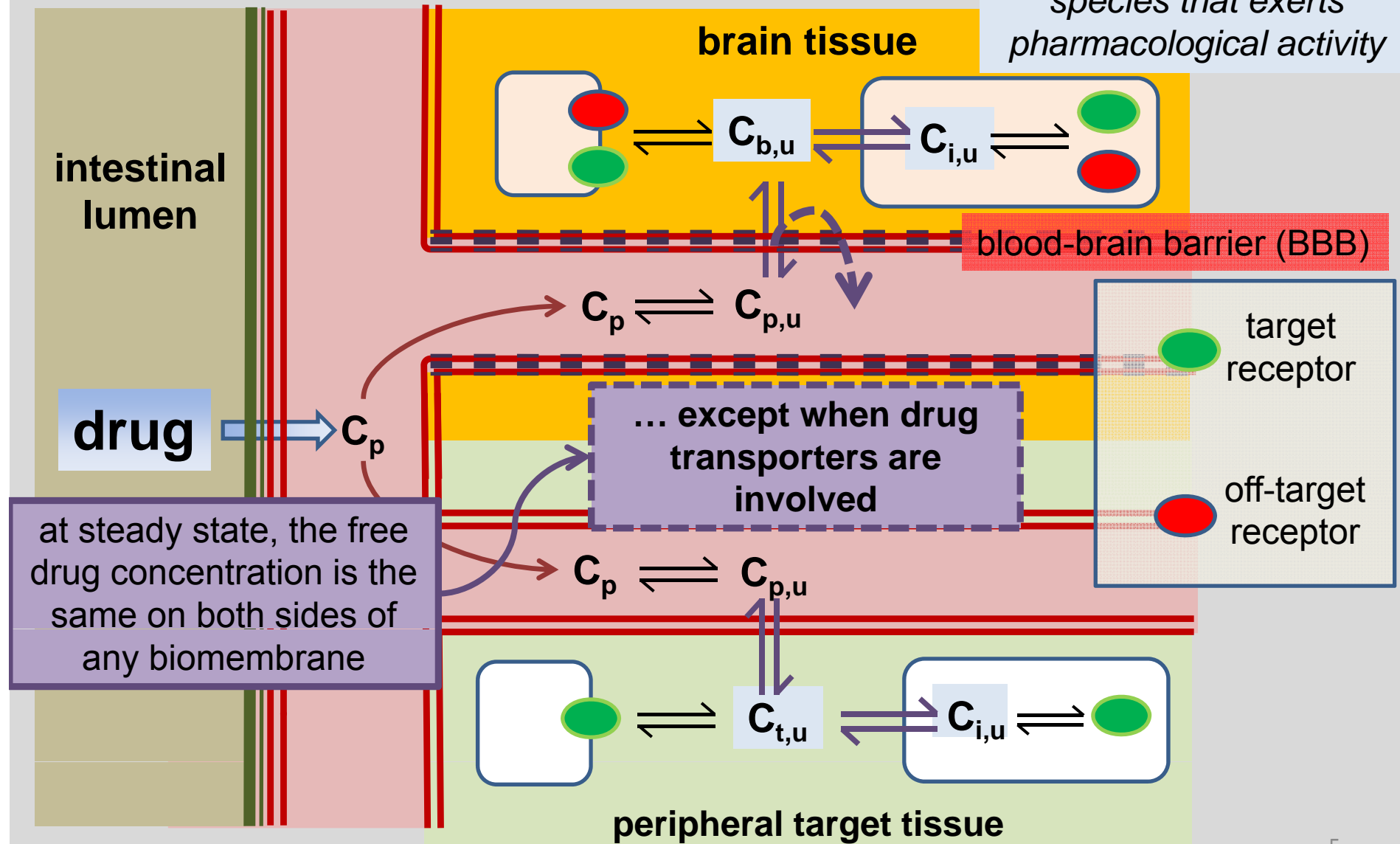


Cole *et al* (2012) *Xenobiotica* 42, 11
Wager *et al* (2012) *Expert Opin. Drug Metab. Toxicol.* 8, 531



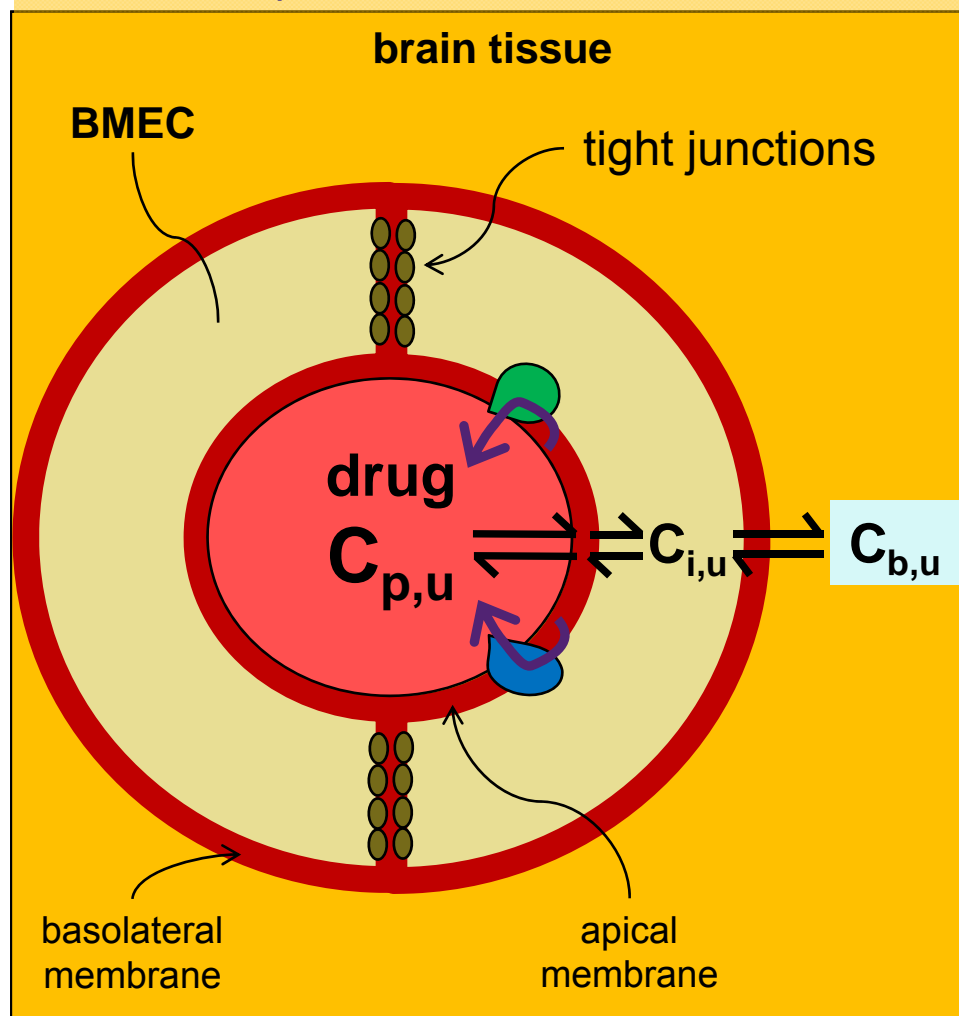
disposition of orally administered drug and the free drug hypothesis

the free drug concentration at the site of action is the species that exerts pharmacological activity



the blood brain barrier (BBB)

schematic representation of a brain microvessel



- ❑ tight junctions between adjacent brain microvascular endothelial cells (BMECs) restrict paracellular movement
- ❑ BMEC apical membrane contains ATP-dependent active **efflux transporters**, including **P-gp** and **BCRP** (ABC family)
- ❑ compounds that are transporter substrates are subject to efflux from within the apical membrane and / or cytoplasm of BMEC against a concentration gradient
- ❑ for a drug that undergoes active efflux, $C_{b,u} < C_{p,u}$ at steady state, representing **CNS restriction**

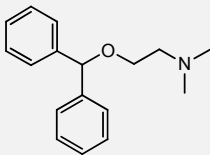
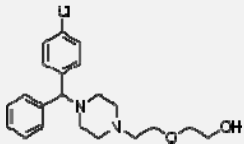
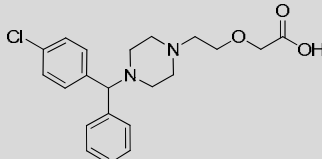
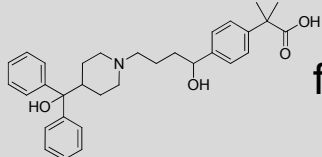
Schinkel (1999) *Adv. Drug Delivery Rev.* 36, 179
 Hammarlund-Udenaes *et al.* (2008) *Pharm. Res.* 25, 1737
 Fagerholm (2007) *Drug Discovery Today* 12, 1076
 Doan *et al.* (2002) *J. Pharmacol. Exp. Ther.* 303, 1029

examples of CNS restriction reducing CNS AEs



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1. histamine H1 antagonists for treatment of allergic reactions

Drug	clinical dose (mg)	somnolence	P-gp substrate	effect of KO on brain penetration *
 diphenhydramine	25 - 50	yes	no	1.0
 hydroxyzine	25	yes	no	1.2
 cetirizine	5 - 10	no	yes	4.4
 fexofenadine	60	no	yes	~ 3

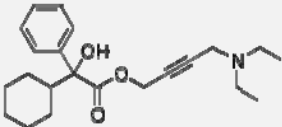
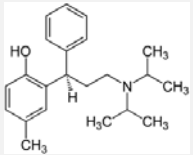
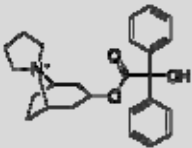
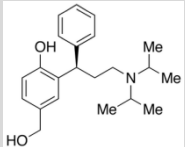
Increasing CNS restriction

Chishty *et al.* (2001) *J. Drug Targeting* 9, 223
Chen *et al.* (2003) *Drug Metab. Dispos.* 31, 312
Tahara *et al.* (2005) *Drug Metab. Dispos.* 33, 963

* effect of P-gp mouse KO on brain penetration expressed as (brain:plasma concentration ratio in KO) / (brain:plasma concentration ratio in wild type)

examples of CNS-restriction reducing CNS AEs

2. antimuscarinic agents for treatment of overactive bladder

Drug	clinical dose (mg)	somnolence	P-gp substrate	unbound brain:plasma ratio ($K_{p,u,u}$)
 oxybutynin	5-30	yes	no	3.3
 tolterodine	4	yes	no	0.23
 trospium	20 - 60	no	yes	0.01
 fesoterodine (5-HMT)	4 - 8	no	yes	0.04

Increasing CNS restriction

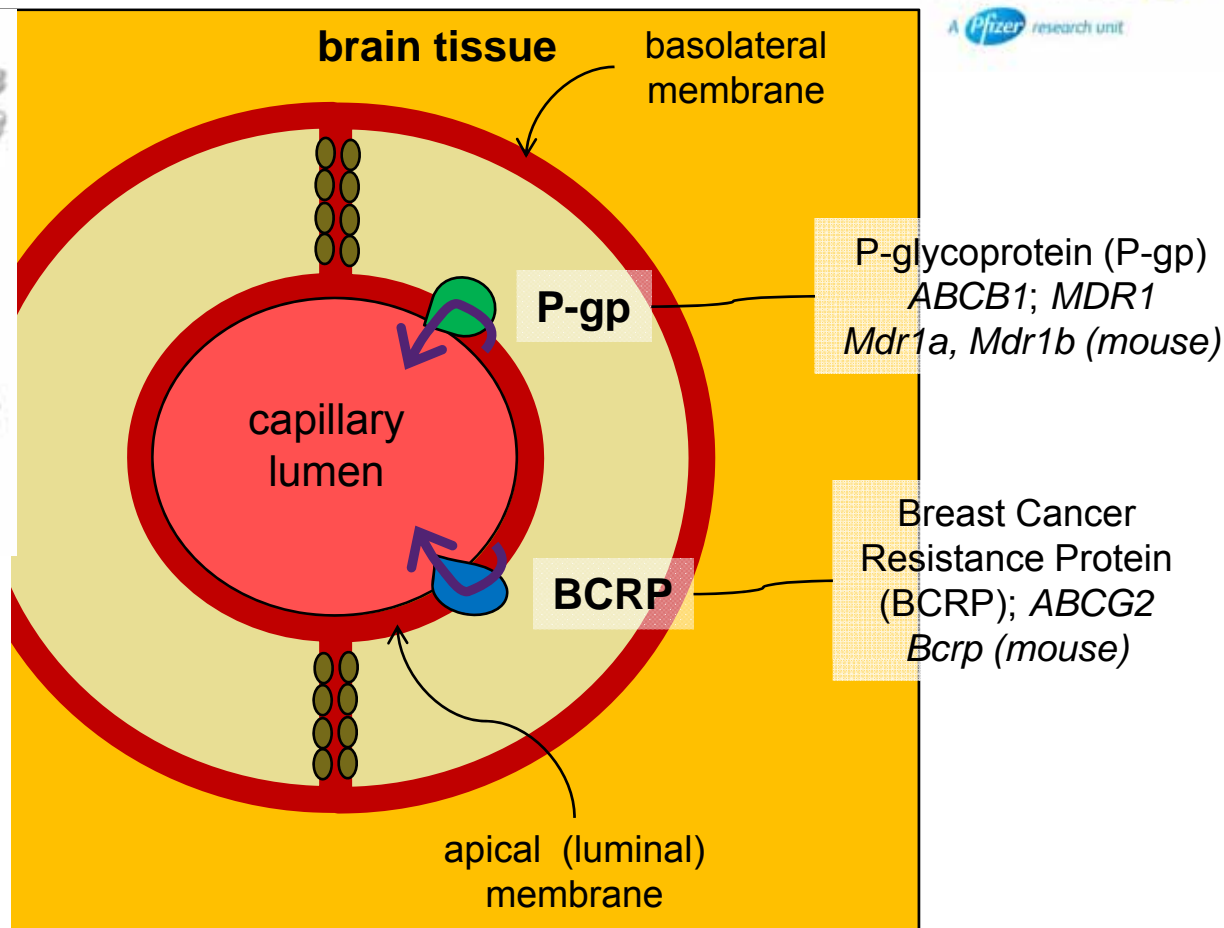
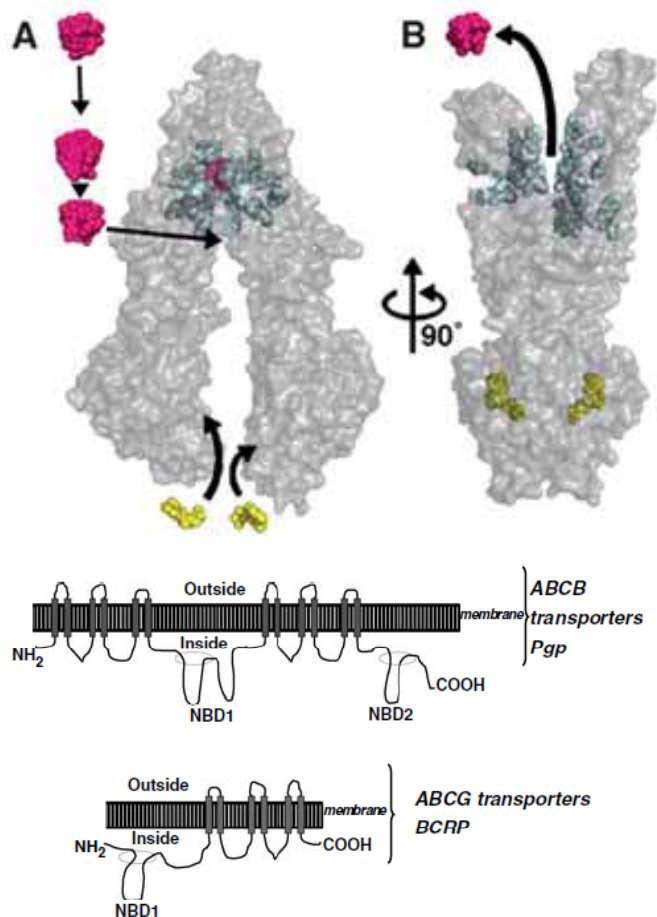
efflux transporters in the BBB



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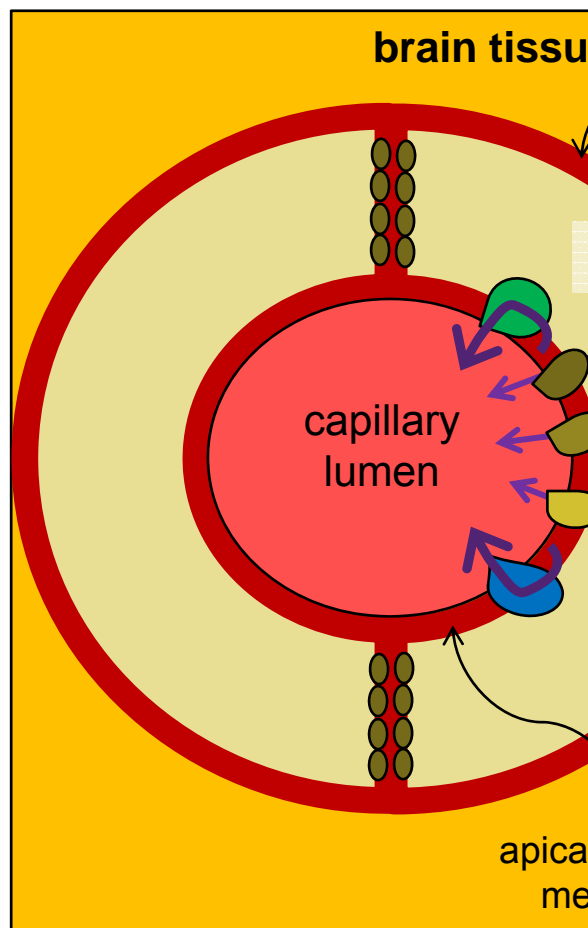
P-glycoprotein (P-gp)



- P-gp and BCRP are the best characterised efflux transporter proteins of the BBB

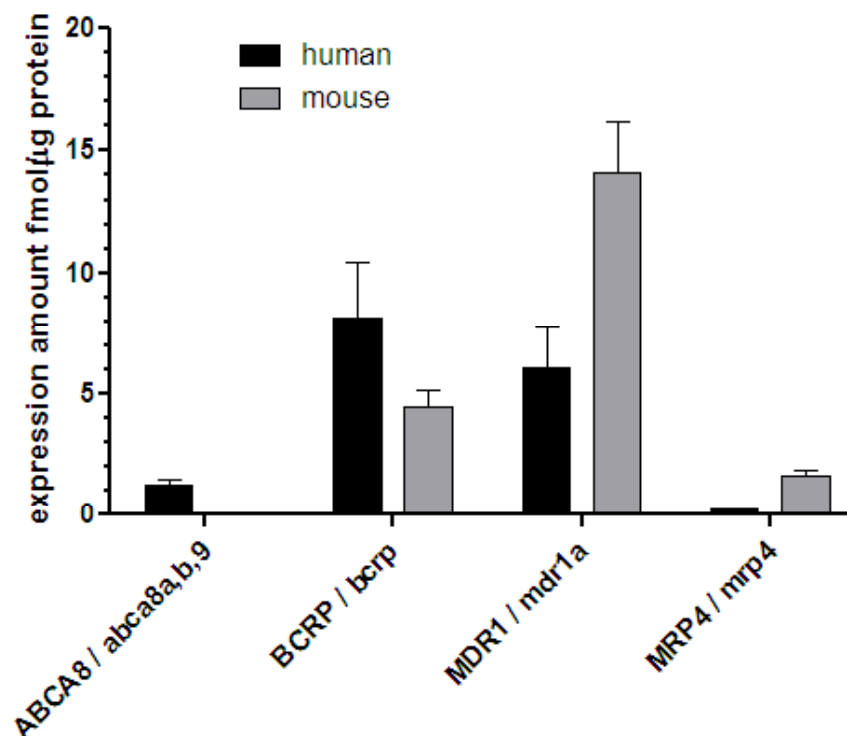
Schinkel *et al.* (1996) *J. Clin. Invest.* 97, 2517
Uchida *et al.*, (2011) *J. Neurochem.* 117, 333

efflux transporters in the BBB



detection of ABC transporters in human BMEC	
transporter	mRNA
MRP1	✓
MRP2	✗
MRP3	✗
MRP4	✓
MRP5	✓
MRP6	✗
P-gp	-
BCRP	-

expression of drug transporters in human and mouse brain microvessels (quantitative proteomics: Uchida et al., 2011)



- expression of other ABC family efflux transporters in human has been described, including MRP (ABCC family)

Miller *et al.* (2000) *Mol. Pharmacol.* 58(6), 1357
 Dauchy *et al.*, (2009) *Biochem. Pharmacol.* 77, 897
 Nies *et al.*, (2004) *Neurosci.* 129, 349
 Chaves *et al.*, (2014) *Curr. Pharmaceut. Design* 20, 1450
 Shawahna *et al.*, (2011) *Mol. Pharmaceutics* 8, 1332

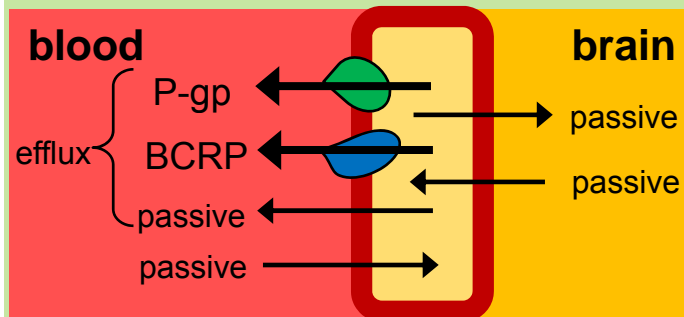
- expression of BCRP and P-gp appears to be much higher than MRP in BBB of human
- functional significance of MRP is not well established

role of P-gp and BCRP in the BBB

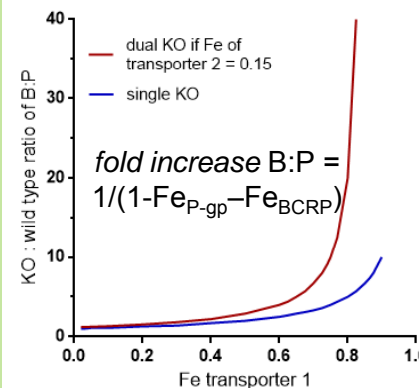


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- the roles of P-gp and BCRP in limiting brain penetration are well established
- broad range of drugs are P-gp and/or BCRP substrates e.g. *imatinib, indinavir, prazosin, topotecan, digoxin, loperamide*
- experiments in Mdr1 /Bcrp KO mice demonstrate the roles of P-gp and BCRP in CNS restriction
- multiple KO of P-gp and BCRP can result in greater than additive fold increases in B:P concentration ratios due to contribution of each transporter to net efflux clearance

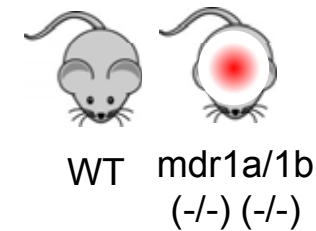


efflux clearance via P-gp and BCRP
expected to be additive

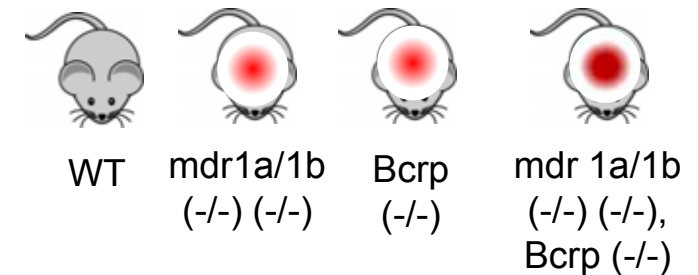


- comparable levels of BCRP and P-gp expressed in human brain microvessels

CNS restriction in human could be maximised in dual P-gp + BCRP substrates



**P-gp substrate
brain:plasma
concentration ratio
(B:P) in KO > WT**

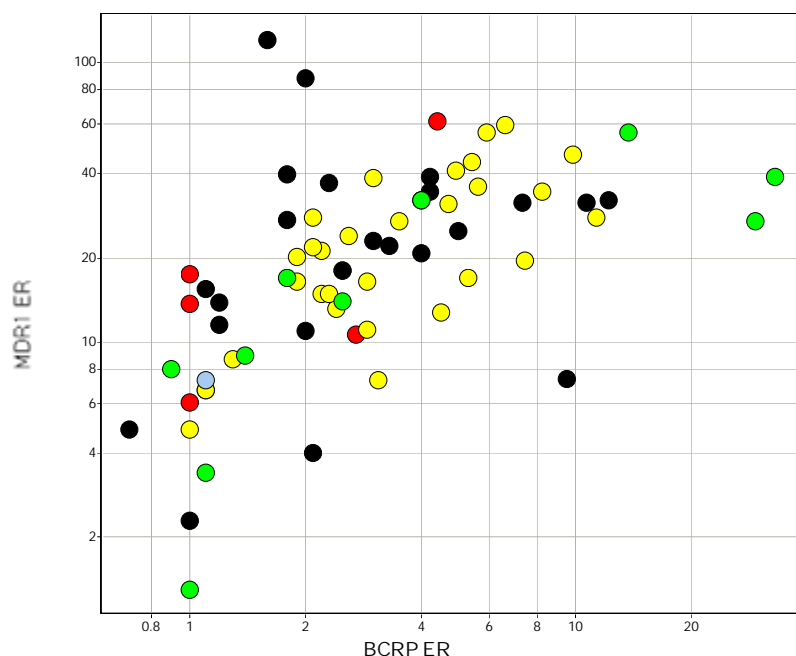
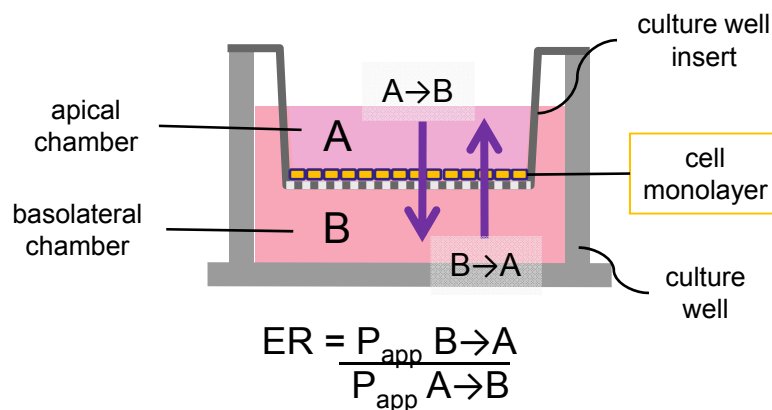


**efflux transporter dual substrate
(e.g. prazosin, lapatinib)
B:P in triple KO >> WT**

Doran et al (2005) Drug Met. Disp. 33,165
Enokizono et al (2008) Drug Met. Disp. 36,995
Zhou et al (2009) Drug Met. Disp. 37,946
Polli et al (2009) Drug Met. Disp. 37, 439
De Vries et al (2007) Clin. Cancer Res. 13, 6440
Kusuhara & Sugiyama (2009) Drug Metab. Pharmacokinet. 24, 37
Kodaira et al (2010) J. Pharm. Exp. Ther. 333, 788
Kalvass et al., (2013) Clin. Pharm. Ther. 94, 80

- Targeting P-gp and BCRP in drug discovery programmes is a pragmatic approach to achieving CNS restriction
- **In vitro and in vivo laboratory approaches to evaluating CNS penetration**

in vitro transporter and permeability assays



transwell permeability assays (used at Pfizer)

MDR1-MDCK : P-gp

BCRP-MDCK : BCRP

MDCK-LE : passive permeability

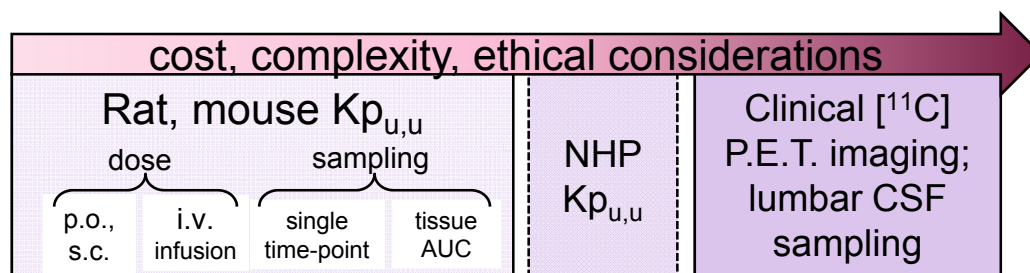
(minimal transporter expression)

- apparent permeability (P_{app}) measured in A to B and B to A directions
- efflux ratio (ER) calculated as ratio of $P_{app} B \rightarrow A$ / $P_{app} A \rightarrow B$
- ER > 1 indicates substrate of the transporter : how much > 1 depends on assay
- sensitivity of ER may be determined with known inhibitors of P-gp (e.g. valspodar) and BCRP (e.g. Ko143) or both (elacridar)
- translation between P-gp and BCRP may be observed and indicate criteria for dual substrates

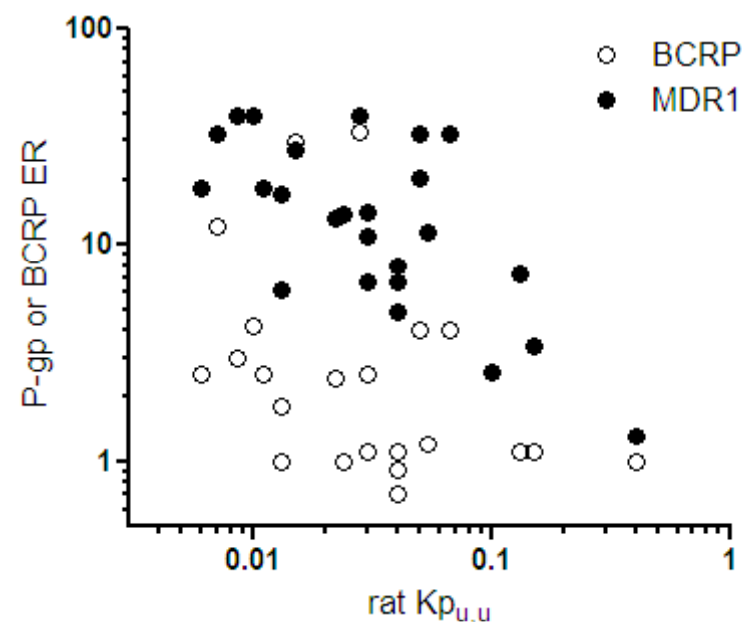
in vivo brain penetration assessment

- Dosing (rodent) by i.v. infusion; p.o., s.c., i.p., aiming to achieve steady state distribution
- Tissue sampling: terminal brain, plasma & cerebrospinal fluid (CSF), single time-point or composite time-course → AUC
- plasma and brain concentrations and drug binding measured in each tissue → unbound concentrations ($C_{b,u}$ and $C_{p,u}$)
- ratio of $C_{b,u} : C_{p,u} = K_{p,u}$ represents pharmacologically relevant brain penetration

- Preclinical translation between in vitro ER and in vivo $K_{p,u}$ may be established for a chemical series
- quantitative importance of BCRP in rat not yet clear
- brain distribution in rat *may* be lower than in human (e.g. verapamil, altanserin)
- Relatively few means of confirming CNS distribution in human



relationship between in vitro ER (transwell



Di, L. *et al.* (2008) *Expert Opin. Drug Discovery* 3, 677
 Di, L. *et al.* (2013) *J. Med. Chem.* 56, 2
 Maurer *et al.* (2005) *Drug Metab. Dispos.* 33, 175

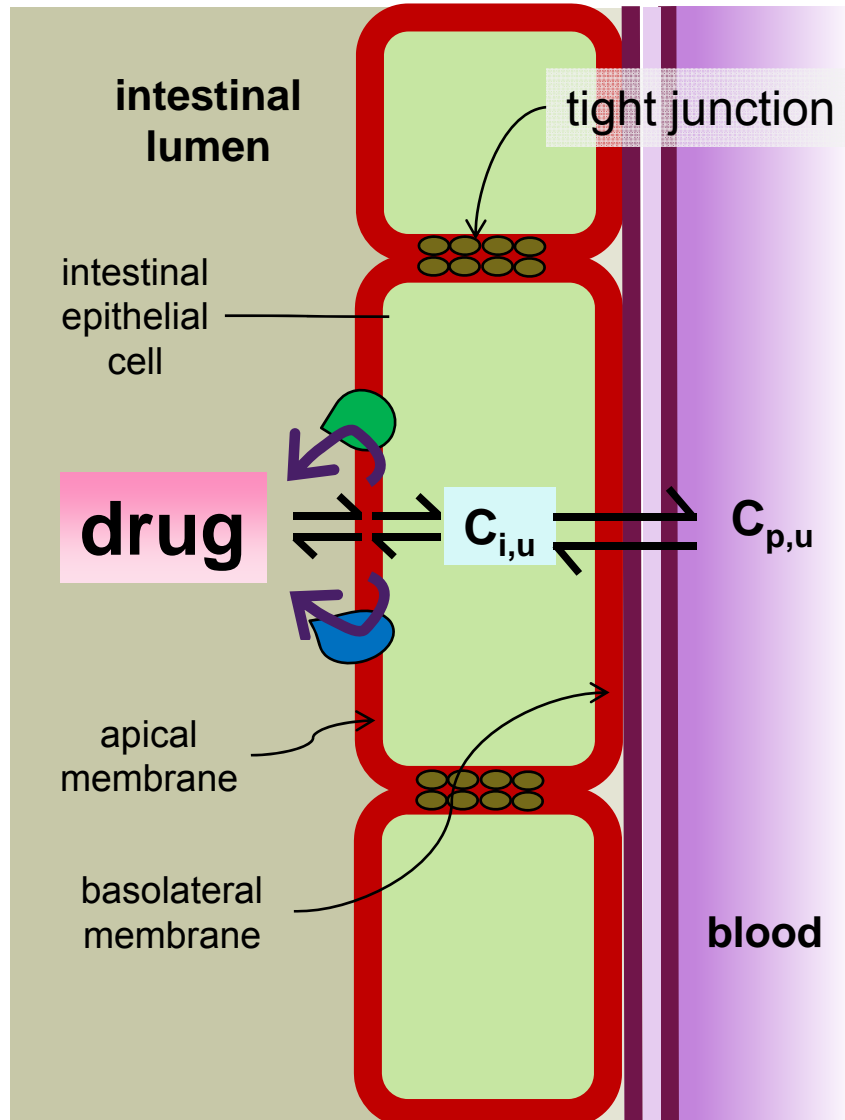
Friden, M. *et al.* (2009) *J. Med. Chem.* 52, 6233
 Bauer, M. *et al.* (2012) *Clin. Pharmacol. Ther.* 91, 227
 Syvaenen, S. *et al.* (2009) *Drug Metab. Dispos.* 37, 635

risks in targeting transporters

- absorption in the GI tract:
 - risk of low bioavailability or non-linear dose vs. exposure relationship
- drug-drug interactions (DDI) altering CNS restriction
- variation in transporter activities in patient populations due to age, disease & polymorphisms

risks in targeting transporters: drug absorption

schematic representation of the intestinal epithelium

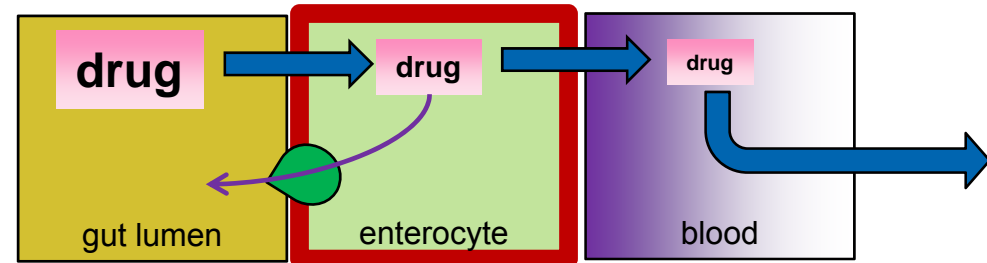


- ❑ intestinal epithelium acts as a barrier to drug absorption:
 - tight junctions between adjacent enterocytes
 - efflux transporters on enterocyte apical membrane
 - efflux transporters could affect absorption of drugs:
 - limited absorption if efflux > passive influx
 - non-linear dose-exposure relationship as transporter saturates
- ❑ high P-gp/BCRP ER does not always translate to poor oral absorption or non-linear dose vs. exposure
- ❑ quantitative importance of P-gp in drug absorption may be questionable

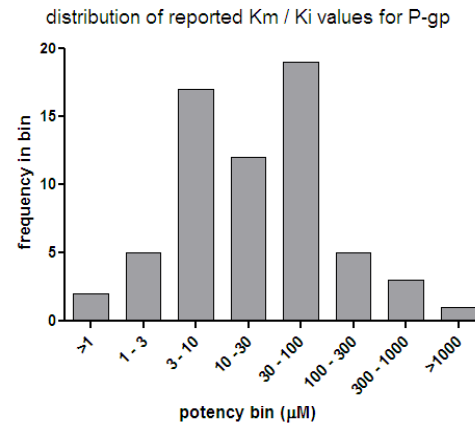
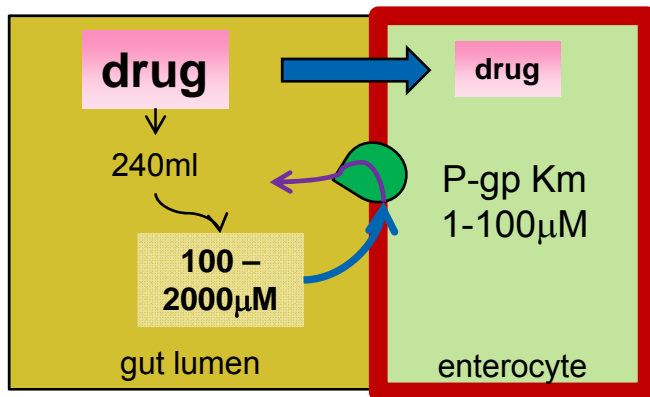
risks in targeting transporters: drug absorption

quantitative importance of P-gp in drug absorption may be limited by:

- high concentration gradient favouring high passive flux across enterocytes
- sink effect: continuous removal of drug from site of absorption by blood flows
- intrinsic permeability may provide passive flux of sufficient magnitude to overcome influence of P-gp



- at clinical therapeutic doses, high intraluminal gut concentration likely to saturate transporters in many cases: reduces probability of non-linear bioavailability with dose



dependent on the balance of:

- transporter kinetics
- drug solubility
- passive permeability

Cole, S. *et al.* (2012) *Xenobiotica* 42, 11
Tachibana, T. *et al.* (2012) *Pharm. Res.* 29, 651
Lin, J. H. (2004) *Drugs Today* 40, 5

Hsu *et al* (1998) *Clin. Pharmacokinet.* 35(4), 275
Peng, B. *et al.* (2005) *Clin. Pharmacokinet.* 44, 879
Chiou *et al* (2001) *Int. J. Clin. Pharm. Ther.* 39, 93

many efflux transporter substrates are capable of being well absorbed and some may display dose-proportional exposure

compound	F %	Fa	clinical dose range (mg)	dose/exposure relationship
fexofenadine	~33	0.3	30 – 180	linear 20 – 240mg
cetirizine	~60	0.5	2.5 - 10	linear 5 – 60mg
digoxin	55 - 79	0.55	0.125 – 0.75	linear 0.25 – 1mg
imatinib	98	~1	400	linear 25 – 1000mg
sunitinib	-	~1 (rat)	37.5 - 50	linear 50 – 350mg
indinavir	63	0.8	800	supraproportional 200 - 800mg
saquinavir	4 *	-	600	supraproportional 75 - 600mg
ritonavir	66-75	-	600	supraproportional 100 - 1000mg
nelfinavir	>78	-	750	supraproportional 300 - 750mg
talinolol	55	>0.5	100	supraproportional 25 - 400mg

dual substrate

P-gp substrate

BCRP substrate

first-pass metabolic extraction may account for some reductions in F%

saturation of P-gp may underlie non-linearity

* saquinavir has high first pass extraction (CL ~ hepatic blood flow)

- interplay of CYP-mediated metabolism (extraction in liver and GI tract) and transporter effects
- often difficult to delineate contributions of each to limiting bioavailability

Cole, S. *et al.* (2012) *Xenobiotica* 42, 11
Williams & Sinko (1999) *Adv. Drug Delivery Rev.* 39, 211
Robbins *et al.* (1998) *Biopharm Drug Dispos.* 19, 455
Peng, B. *et al.* (2005) *Clin. Pharmacokinet.* 44, 879
Chiou *et al.* (2001) *Int. J. Clin. Pharm. Ther.* 39, 93
Tubic *et al.* (2006) *Pharm. Res.* 23, 1712

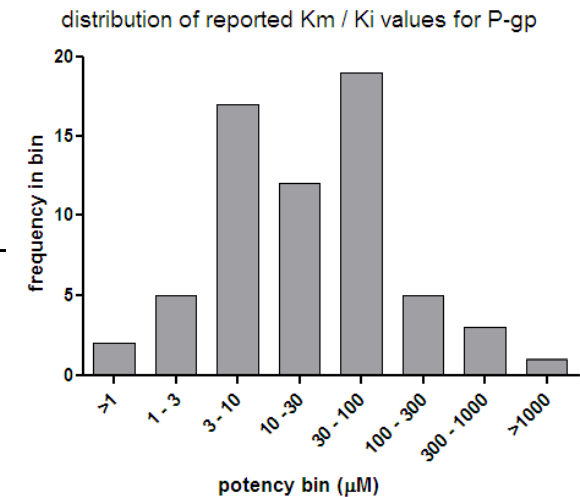
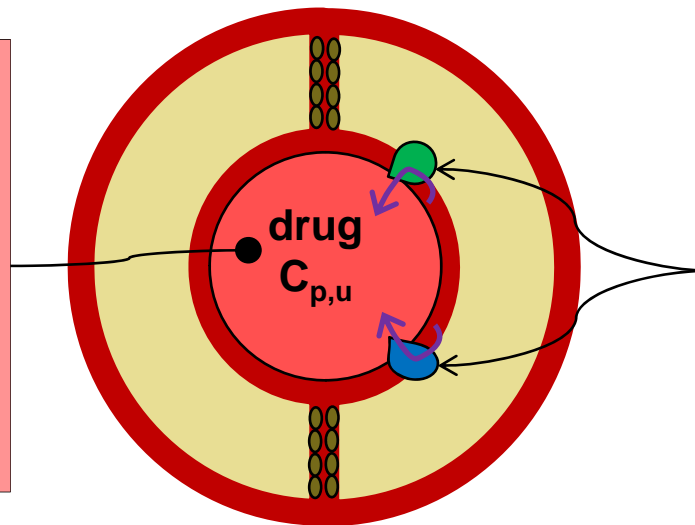
risks in targeting transporters: DDI at BBB



$C_{p,u} < 1-10 \mu\text{M}$

inhibitors of transporters unlikely to reach high enough concentrations to cause DDI

saturation of transporters by substrates unlikely



- clinical relevance of P-gp inhibition to CNS effects of loperamide at recommended doses thought to be low
- coadministration of the P-gp inhibitor tariquidar with verapamil led to maximal 2.7x increase in ^{11}C -verapamil distribution into the brain
- cyclosporin infusion increased brain: blood AUC of ^{11}C -verapamil by 88%
 - relatively high doses of precipitant drugs are capable of causing increases in CSF and/or brain : plasma concentration ratios ($\sim 2-6$ fold \uparrow)

• **ITC review (Kalvass et al., 2013) concluded that there is low risk of DDI at the BBB**

Vandenbossche et al., (2010), J. Pharm. Pharmacol. 62, 401
 Bauer et al., (2012) Clin. Pharm. Ther. 91, 227
 Eyal et al., (2009) Pharmacol. Ther. 123, 80
 Sasongko et al., (2005) Clin. Pharm. Ther. 77, 503

Fenner et al., (2010) Clin. Phar. Ther. 85(2), 173
 Di et al., (2008) Expert Opinion Drug Disc. 3, 677
 Lin (2004) Drugs Today 40, 5
 Tachibana et al., (2012) Pharm. Res. 29, 651
 Kalvass et al (2013) Clin. Pharm. Ther. 94, 80

risks in targeting transporters: variation in patient populations due to age, disease & genetic variation

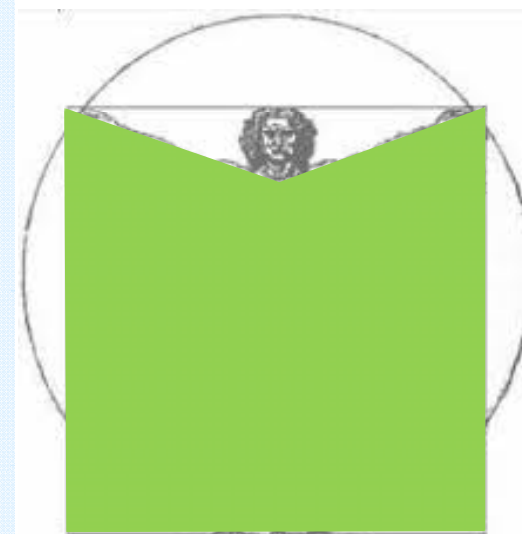
- several polymorphisms of the human MDR1 (P-gp) and BCRP genes exist
 - → changes in transporter activity / expression
 - → possible effects on drug disposition
 - MDR1: e.g. SNP C3435T associated with reduced expression in duodenum
 - BCRP: e.g. 421C>A polymorphism → reduced BCRP expression
 - MDR1 gene polymorphisms:
 - lack of consistency in observed effects
 - unclear if drug disposition changes are polymorphism related
 - BCRP 421C>A polymorphism increased AUC of rosuvastatin and atorvastatin and of sunitinib in renal carcinoma patients
 - Changes in P-gp function in ageing, Alzheimer's disease & Parkinson's disease inferred from CNS restriction of [¹¹C]-verapamil in clinical PET imaging studies
 - Patient populations may possess different degrees of transporter-dependent CNS restriction (e.g. demographic groups; disease states)
- **targeting both P-gp and BCRP may reduce the risk of changes in CNS restriction due to these factors due to functional redundancy**

Marzolini et al (2004) Clin. Pharm. Ther. 75, 13
Lee et al., (2007) Drug Met. Disp. 35, 623
Hoffmeyer et al., (2000) P.N.A.S. 97, 3473
Skarke et al., (2003) Pharmacogenetics 13,651
Cascorbi (2006) Pharmacol. Ther. 112, 457
Keskitalo et al (2009) Clin. Pharmacol. Ther. 86, 197

Mizuno et al (2012) Drug Metab. Pharmacokinet. 27, 631
Bartels et al (2009) Neurobiol. Aging 30, 1818
Toornvliet et al. (2006) Clin. Pharmacol. Ther. 79, 540
van Assema et al. (2012) Brain 135, 181
Bartels et al. (2008). J. Neural Transm. 115, 1001

conclusion

- ❑ **CNS restriction of peripherally targeted drugs may improve their safety and tolerability**
- ❑ **targeting P-gp and BCRP in human is a pragmatic approach to maximising CNS restriction**
- ❑ **in vitro and in vivo assays are available to design compounds with CNS restriction**
- ❑ **risks of this approach include:**
 - translation of preclinical to clinical
 - poor and/or variable GI absorption – low risk for highly permeable and soluble compounds
 - transporter-mediated drug interaction – low risk at 'normal' clinical doses
 - variation in transporter activity in the human population (age, polymorphisms) – targeting both P-gp and BCRP may minimise risk



acknowledgements



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