A REVIEW ON THE RELATION BETWEEN THE BRAIN-SERUM CONCENTRATION RATIO OF DRUGS AND THE INFLUENCE OF P-GLYCOPROTEIN

Thomas Broeng Ejsing[1], Niels Morling[2] and Kristian Linnet[1]*

[1]*Section of Forensic Chemistry and* [2]*Section of Forensic Genetics, Institute of Forensic Medicine, Faculty of Health Sciences, University of Copenhagen, Denmark*

SUMMARY

This overview on the brain-serum relationship for drugs illustrates the importance of the drug transporter P-glycoprotein at the blood-brain barrier. Generally, an inverse relationship exists between the magnitude of the brain-serum ratio and the influence of P-glycoprotein. Concerning the pharmacogenomics of P-glycoprotein, no clear effect of single nucleotide polymorphisms (SNPs) has been demonstrated in humans.

KEY WORDS

blood-brain barrier, drugs, lipophilicity, pharmacogenomics, P-glycoprotein, water-octanol partition coefficient

* Author for correspondence:
Kristian Linnet, M.D.
Section of Forensic Chemistry
Institute of Forensic Medicine
Faculty of Health Sciences
University of Copenhagen
Frederik V's Vej 11
DK-2100 Copenhagen, Denmark.
e-mail: linnet@post7.tele.dk

INTRODUCTION

The blood-brain barrier (BBB) is a major impediment to the entry of many therapeutic drugs into the brain, and during the last decade it has become clear that multispecific, xenobiotic transporters play an important role in BBB function. The prime member of this group of transporters is P-glycoprotein (P-gp). P-gp exerts an important influence on the brain concentrations of many types of drugs, such as opioids (e.g. morphine and methadone), antidepressants (e.g. amitriptyline and nortriptyline), antipsychotics (e.g. olanzapine and risperidone), the anthelmintic agent ivermectin, the cardiac glycoside digoxin, HIV protease inhibitors (e.g. ritonavir and nelfinavir) and the new generation of antihistamines (e.g. cetirizine and loratidine) /1-5/. Here, we present a brief overview of the role of P-gp at the BBB with emphasis on the quantitative importance of P-gp with regard to drug distribution over the BBB and on the pharmacogenomics of P-gp.

STRUCTURE, FUNCTION AND PHYSIOLOGY OF P-GLYCOPROTEIN

P-glycoprotein is the product of the human *MDR1* gene (recently renamed *ABCB1*) and was initially discovered for its role in the development of multi-drug resistance (MDR) of cancer cells /6/. P-gp is a large, glycosylated membrane protein localized primarily in the plasma membrane of the cell belonging to the ABC transport protein family (ATP-binding cassette proteins) /7/. It contains two homologous, but not identical, parts joined together by a short linker region /8/. Each part comprises six transmembrane α-helices and an ATP-binding site (Fig. 1). The 12 transmembrane segments fold together to form a barrel-like structure that traverses the plasma membrane. The two ATP-binding sites are located at the inner side of the plasma membrane, where hydrolysis of ATP provides the energy necessary for drug transport out of the cell /7/. A suggested model of its function is that lipophilic drugs are intercepted when they move through the lipid cell membrane and are flipped from the inner to the outer leaflet and into the extracellular space /9/.

P-glycoprotein is located in the luminal membrane of endothelial cells of brain capillaries, and it limits the accumulation of drugs in the brain by actively pumping them back into the blood (Fig. 2) /10/. High levels of expression of P-gp have also been observed in the brush

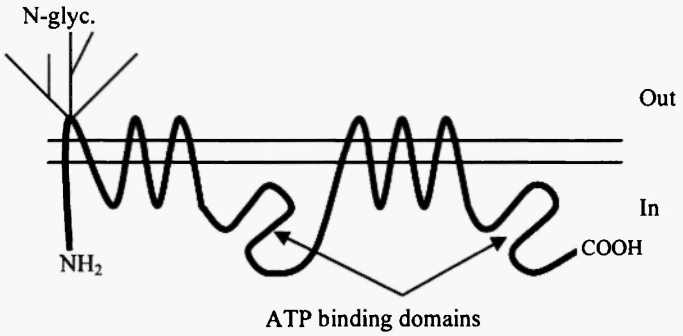

Fig. 1: Two dimensional representation of human P-glycoprotein. The 12 transmembrane segments fold together to form a three-dimensional barrel-like structure in the membrane. N-glyc = the N-linked glycosylation 'trees' that are found on the first extracellular loop. Modified from /14/.

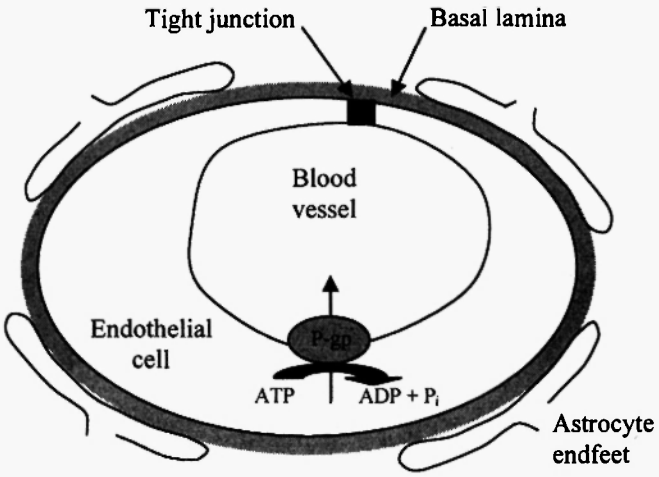

Fig. 2: P-glycoprotein at the blood-brain barrier. Modified from /14/.

border of the proximal renal tubule, on the surface of the biliary hepatocytes, and in the mucosa of the small and large intestines /7/. In these locations, P-gp excretes xenobiotics and endogenous metabolites out of the body into urine, bile or the intestinal lumen.

There is some species variation of P-gp. Rodents possess two types, coded by the *MDR1a* and *MDR1b* genes, which correspond to the human *MDR1* form. Additionally, there exists a *MDR3* form, which is supposed to be primarily a phospholipid transporter /11/.

There is currently very little structural information available for P-gp. In particular, the number and locations of the drug binding sites within P-gp have been the object of much speculation /12/, and as many as four drug binding sites have been reported /13/. Numerous studies have shown that P-gp substrates tend to be of amphipathic nature /14/. Therefore, the concentration of the P-gp substrates in the membrane can be far higher than in solution /15-17/. This substrate accumulation in the membrane may allow P-gp to recognize a wide spectrum of substrates with low affinity using a non-specific hydrophobic binding pocket, as suggested by Pawagi *et al.* /18/. This could be an explanation for the lack of clearly defined binding properties of substrates /19/. Hence, there may be no clear difference between substrates and non-substrates, but instead a gradual transition from substrate to non-substrate.

QUANTITATIVE INFLUENCE OF P-GLYCOPROTEIN AT THE BLOOD-BRAIN BARRIER

Prediction of drug penetration into the brain has been intensely investigated for decades, and some studies have shown that the water-octanol partition coefficient (K_{ow}) is related to the ability of compounds to cross the endothelial cell membranes at the BBB /20,21/. In the former study, an approximately linear relationship was found between the log of K_{ow} divided by the square root of the molecular weight (MW), i.e. $\log(K_{ow}/MW^{1/2})$, and the log of the BBB permeability coefficient for a number of compounds. Analogously, we considered the relationship between $\log(K_{ow}/MW^{1/2})$ and the brain-serum concentration ratio, and were interested in delineating the impact of P-gp in relation to the physical-chemical properties of drugs. We performed a literature search and found 57 drugs and metabolites that had significantly different brain-serum concentration ratios in P-

gp knockout mice (KO mice) and wild type (WT) mice (Table 1). As a quantitative measure of the impact of P-gp at the BBB, we focussed on the brain-serum concentration ratio in KO mice divided by the brain-serum concentration ratio in WT mice (KO/WT ratio). K_{ow} values for 46 of the 57 above-mentioned drugs were found, and log ($K_{ow}/MW^{1/2}$) was calculated. Figure 3 shows plots of brain-serum ratios (ordinate) against the log($K_{ow}/MW^{1/2}$) value (abscissa) for KO (left) and WT (right) mice, respectively. We observed a considerable scatter with slightly positive coefficients of correlation amounting to 0.092 and 0.046 for KO and WT mice, respectively. The coefficients, however, were not significantly different from zero. Thus, the log($K_{ow}/MW^{1/2}$) value was not significantly correlated to the brain-serum concentration ratio for the present set of drugs, irrespective of whether P-gp was present (WT mice) or absent (KO mice). However, various additional factors influence this ratio, e.g. protein binding of drugs in serum. A better measure for the distribution over the BBB might be the ratio of cerebral to *free* drug concentration in serum.

As an alternative to the above-mentioned plots, we plotted the KO/WT ratios (ordinate) of the 57 drugs in Table 1 against the brain-serum ratios (abscissa) of the KO and WT mice (Fig. 4 left and right, respectively). It is apparent that there was an inverse relationship between the effect (KO/WT ratio) of P-gp and the brain-serum ratios in WT mice ($r = -0.34$; $p < 0.05$). Drugs with high brain-serum ratios tended to have low KO/WT ratios, and vice versa. For example, if we consider psychotropic drugs, chlorpromazine (brain-serum ratio 23 in WT mice) had a KO/WT-ratio of 1.3, whereas risperidone (brain-serum ratio 0.52 in WT mice) had a KO/WT ratio of 13. Taking the group of drugs with brain-serum ratios exceeding one in WT mice, the average KO/WT ratio was 2.63 (SE 0.47, $n = 21$) compared to 8.98 (SE 1.46, $n = 35$) for those drugs that have brain-serum ratios below one in WT mice.

An explanation for the relationships outlined above may be that drugs with high brain-serum ratios traverse the plasma membrane too quickly for the P-gp mediated efflux to keep pace with the influx. On the other hand, low brain-serum ratios make it possible for P-gp to mediate a drug efflux of relatively larger importance at the BBB. This implies that psychoactive drugs, which tend to have high brain-serum ratios, are likely to be less influenced by variations in P-gp function. In contrast, for drugs with low brain-serum ratios, e.g. risperidone, inter-

TABLE 1

Drugs included in the analysis

Drug	Brain-serum ratio		KO/WT ratio	log K_{ow}	MW	Reference
	KO mice	WT mice				
Amiodarone*	1.7	0.08	21	6.64	645	/24/
Amprenavir*	1.1	0.3	3.7	–	–	/3/
Asimadoline@	6.7	0.7	9.1	–	–	/25/
Buspirone*	2.0	1.6	1.3	3.39	386	/24/
Caffeine*	1.1	1.0	1.1	-0.08	194	/24/
Carbamazepine*	0.81	0.76	1.1	2.67	236	/24/
Carebastin*	1.47	0.33	4.2	–	–	/26/
Cerivastatin*	0.049	0.014	3.6	–	–	/27/
Chlorpromazine*	29	23	1.3	3.36	319	/24/
Citalopram*	9.7	5.1	1.9	0.74	324	/24/
Clozapine*	6.6	4.1	1.6	3.28	327	/24/
Compound A*	9.85	1.11	8.9	–	–	/28/
Compound D*	1.03	0.05	20.7	–	–	/28/
Compound X*	0.67	0.15	4.5	–	–	/28/
Cyclobenzaprine*	16	12	1.4	4.42	275	/24/
Demethylnortriptyline*	13	8.5	1.5	1.18	251	/29/

Drug	Brain-serum ratio		KO/WT ratio	Log K_{ow}	MW	Reference
	KO mice	WT mice				
Dexamethasone@	0.256	0.156	1,6	1.83	393	/30/
Diazepam*	2.3	2.0	1 2	2 96	285	/24/
Digoxin@	0.57	0 06	9.5	1.26	781	/2/
Do•etaxe@	1.7	0.29	5.9	–	–	/31/
E-10-OH-nortriptyline*	3.1	0.5	6.4	2.1	279	/29/
Ebastine*	3.73	1.65	5.3	2.78	470	/25/
Fexofenadine@	0.13	0.04	3.3	–	–	/32/
Fluoxetine*	18	12	1.5	1.83	309	/24/
Fluvoxamine*	14	6.1	2.3	1.21	318	/24/
Haloperidol*	18	13	1.4	2.57	376	/24/
Hydrocodone*	4.5	2 1	2.1	1.2	299	/24/
Indinavir@	0.7	0 2	3.5			/3/
@	0.8	0.08	10	2.79	614	/33/
Loperamide*	5.1	0.55	9 3	4.22	477	/24/
Meprobamate*	0.7	0.42	1.7	0.7	218	/24/
Methylphenidate*	19	12	1.6	-0 28	233	/24/
Metoclopramide*	7.9	1.2	6.6	0.18	300	/24/
Morphine*	0.76	0.46	1.7	0.33	285	/24/

Drug	Brain-serum ratio		KO/WT ratio	Log K_{ow}	MW	Reference
	KO mice	WT mice				
Nelfinavir@	2.7	0.09	29	4	568	/33/
@	2.3	0.08	23			/3/
Nortriptyline*	30	18	1.6	3.11	263	/29/
*	20	11	1.8			/24/
Olanzapine*	2 0	0.9	2.6	2.89	312	/34/
Paroxetine*	7.1	3.3	2 2	1.19	329	/24/
Phenytoin*	0.78	0.63	1.2	2.44	252	/24/
Prednisolone@	0.725	0.225	3.2	1.62	360	/35/
Propoxyphene*	7.6	2 9	2 6	3.66	339	/24/
Quinidine*	10	0 28	36	1.58	324	/24/
R-methadone@	2	0.2	10	3.93	310	/36/
Risperidone*	5.5	0.4	12			/37/
*	5.6	0.4	14			/5/
*	8.0	0.78	10	2.22	410	/24/
9-OH-risperidone*	0.3	0.01	29			/37/
*	3.2	0.3	12			/5/
*	1.0	0.06	17	0.91	426	/24/
Ritonavir*	2.31	0.17	14	–	–	/28/
S-methadone@	5	0.2	25	3.93	310	/36/

Drug	Brain-serum ratio		KO/WT ratio	Log K_{ow}	MW	Reference
	KO mice	WT mice				
Saquinavir[@]	1.2	0.2	6.0	4.51	671	/3/
[@]	0.88	0.13	6.8			/32/
Selegiline*	4.1	3.7	1.1	2.55	187	/24/
Sparfloxacin[@]	2.4	0.7	2.0	0.01	392	/38/
STI-571[@]	0.05	0.005	10	–	–	/39/
Sulpirid*	0.15	0.078	1.9	-1.12	341	/24/
Tacrolimus[@]	16.4	2.73	6.0	3.77	804	/40/
Thiopental*	0.44	0.36	1.2	2.84	242	/24/
Venlafaxine*	7.7	4.2	1.8	2.49	307	/24/
Verapamil*	5.6	0.34	17	3.28	455	/24/
Vinblastine[@]	11	0.85	12.8	3.69	811	/41/
Zolpidem*	0.40	0.29	1.4	1.07	277	/24/

mdr1a, *mdr1a/1b* and CF-1 (spontaneous loss of *mdr1a*) KO mice were included. As only *mdr1a* P-gp is expressed in the rodent blood-brain barrier, all three animal models should be equally good /22,23/.

The log K_{ow} values were taken from either Doran *et al.* /24/ or the homepage of the Sangster Research Laboratories (http://logkow.cisti.nrc.ca/logkow/index.jsp).

* The KO/WT ratio was given in the cited reference.

[@] The KO/WT ratio was calculated from average values given in the cited reference. Where more than one article is cited, the average value from all the cited works was used in the plots

MW = molecular weight; KO = knockout; WT = wild type; K_{ow} = water-octanol partition coefficient.

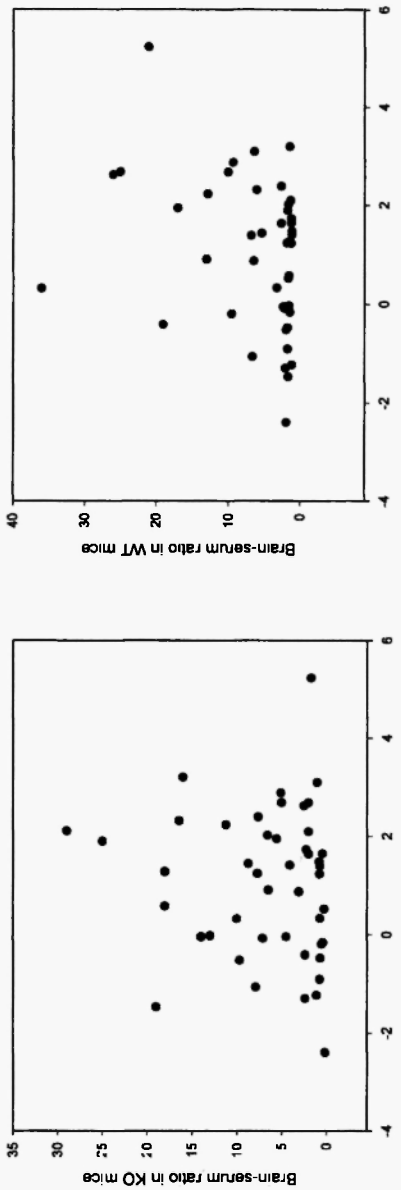

Fig. 3: Left: Brain-serum ratio of knockout (KO) mice plotted against $\log(K_{ow}/MW^{1/2})$. Right: Brain-serum ratio of wild type (WT) mice plotted against $\log(K_{ow}/MW^{1/2})$. MW = molecular weight; K_{ow} = water-octanol partition coefficient. Data and references are shown in Table 1.

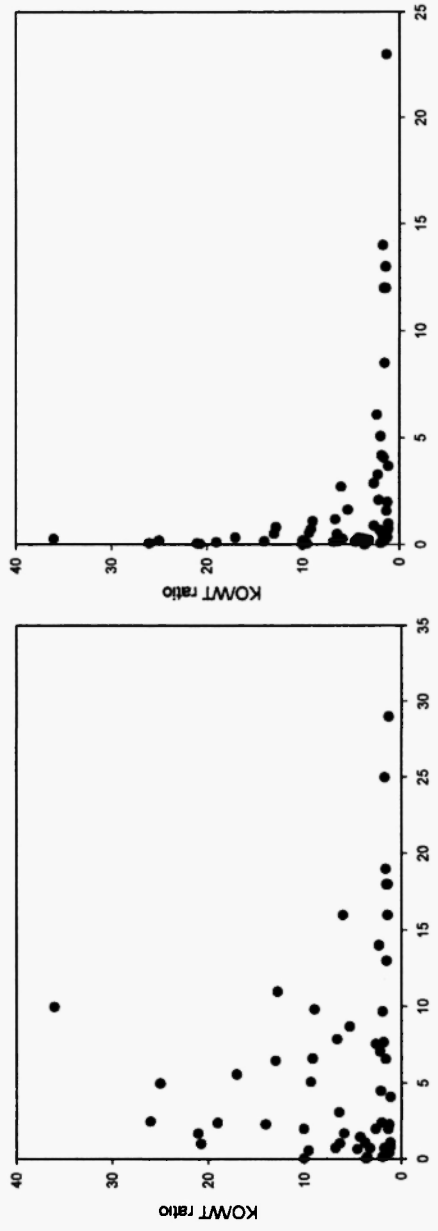

Fig. 4: Left: Knockout (KO)/wild type (WT) ratios plotted against the brain-serum ratios in KO mice. Right: KO/WT ratios plotted against brain-serum ratios in WT mice. K_{ow} = water-octanol partition coefficient. Data and references are shown in Table 1.

individual variations in P-gp expression or function could be of importance with respect to failure of treatment or occurrence of unexpected adverse effects.

PHARMACOGENOMICS OF P-GLYCOPROTEINP

The pharmacogenomics of the human P-gp gene, *MDR1* (*ABCB1*) has been studied intensely in recent years. Since psychoactive drugs are P-gp substrates, variations in P-gp expression and/or function at the BBB could, at least in part, be a factor with regard to inter-individual variation in response or occurrence of side effects at identical drug serum concentrations. The human *MDR1* gene harbours approximately 30 single nucleotide polymorphisms (SNPs) /42/. SNPs in exons 21 and 26 are of particular interest, as these have been associated with differences in P-gp expression in humans /43/. The majority of SNP-related reports are concerned with the silent C3435T SNP of exon 26. This SNP has been associated with both increased and decreased levels of P-gp in the intestines, and with changed absorption patterns for digoxin and fexofenadine /44-46/. With regard to the effect of the C3435T SNP on P-gp at the BBB, few studies are available. In a study concerning depressed patients, Roberts *et al.* /47/ found no difference in serum levels of nortriptyline between C/C, C/T, and T/T genotypes. They did, however, report that there was an increased occurrence of postural hypotension for the patients homozygous for T. In another study, de Luca *et al.* /48/ investigated the involvement of the C3435T SNP in antidepressant-induced mania in depressed patients. The study included 55 patients treated with fluoxetine, fluvoxamine, sertraline, imipramine, moclobemide, venlafaxine, paroxetine, nefazodone, or combination therapy with fluoxetine/fluvoxamine. They found no association, but the large number of different drugs included in the study is a problem, since, for example, sertraline and fluoxetine are at best poor substrates of P-gp /24,49/. Thus, further studies are warranted to elucidate the possible influence of the C3435T SNP at the BBB on the effect of psychotropic drugs. Generally, the majority of studies concerning the effect of P-gp polymorphisms on adverse effects or brain penetration of drugs do not show any significant effects. Interestingly, no loss-of-function mutation has been described for *MDR1* in humans /50/. In collie dogs, on the other hand, lack of functional P-gp occurs frequently. About one-

third of collie dogs have no functional P-gp because of a four base-pair deletion in the gene. When these collie dogs are treated with standard doses of loperamide, they are subject to several neurotoxic side effects, including mydriasis, ataxia, prostration and disorientation /51,52/. Likewise, ivermectin gives rise to neurotoxicity when these dogs are treated for mite infections /53/.

CONCLUSION

The present overview on the brain-serum concentration relationship for a number of important drugs illustrates the importance of the drug transporter P-gp at the BBB. Generally, an inverse relationship exists between the magnitude of the brain-serum ratio and the influence of P-gp, as expressed by the relative difference between brain-serum ratios in KO and WT mice (KO/WT ratios). Although the pharmacogenomics of P-gp has been the focus of much interest, no clear effect of SNPs on the function of P-gp at the BBB has been demonstrated in humans. However, the possible effects of SNPs in the *MDR1* gene is an area that deserves further attention.

REFERENCES

1. Schinkel AH, Smit JJM, van Tellingen O, Beijnen JH, Wagenaar E, van Deemter L, Mol CAA, van der Valk MA, Robanus-Maandag EC, te Riele HPJ, Berns AJM, Borst P. Disruption of the mouse *mdr1a* P-glycoprotein gene leads to a deficiency in the blood-brain barrier and to increased sensitivity to drugs. Cell 1994; 77: 491-502.
2. Mayer U, Wagenaar E, Beijnen JH, Smit JW, Meijer DKF, van Asperen J, Borst P, Schinkel AH. Substantial excretion of digoxin via the intestinal mucosa and prevention of long-term digoxin accumulation in the brain by the mdr1a P-glycoprotein. Br J Pharmacol 1996; 119: 1038-1044.
3. Choo EF, Leake B, Wandel C, Imamura H, Wood AJJ, Wilkinson GR, Kim RB. Pharmacological inhibition of P-glycoprotein transport enhances the distribution of HIV-1 protease inhibitors into brain and testes. Drug Metab Dispos 2000; 28: 655-660.
4. Ejsing TB, Linnet K. Influence of P-glycoprotein inhibition on the distribution of the tricyclic antidepressant nortriptyline over the blood-brain barrier. Hum Psychopharmacol 2005; 20: 149-153.
5. Ejsing TB, Pedersen AD, Linnet K. P-glycoprotein interaction with risperidone and 9-OH-risperidone studied in vitro, in knock-out mice and in drug-drug interaction experiments. Hum Psychopharmacol 2005; 20: 493-500.

6. Juliano RL, Ling V. A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. Biochim Biophys Acta 1976; 455: 152-162.
7. Schinkel AH. The physiological function of drug-transporting P-glycoproteins. Sem Cancer Biol 1997; 8: 161-170.
8. Bosch I, Croop JM. P-glycoprotein structure and evolutionary homologies. Cytotechnology 1998; 27: 1-30.
9. Higgins CF, Gottesman MM. Is the multidrug transporter a flippase? Trends Biochem Sci 1992; 17: 18-21.
10. Bendayan R, Lee G, Bendayan M. Functional expression and localization of P-glycoprotein at the blood brain barrier. Microsc Res Tech 2002; 57: 365-380.
11. Smith AJ, van Helvoort A, van Meer G, Szabó K, Welker E, Szakács G, Váradi A, Sarkardi B, Borst P. MDR3 P-glycoprotein, a phosphatidylcholine translocase, transports several cytotoxic drugs and directly interacts with drugs as judged by interference with nucleotide trapping. J Biol Chem 2000; 275: 23530-23539.
12. Lugo MR, Sharom FJ. Interaction of LDS-751 with P-glycoprotein and mapping of the location of the R drug binding site. Biochemistry 2005; 44: 643-655.
13. Martin C, Berridge G, Higgins CF, Mistry P, Charlton P, Callaghan R. Communication between multiple drug binding sites on P-glycoprotein. Mol Pharmacol 2000; 58: 624-632.
14. Schinkel AH. P-glycoprotein, a gatekeeper in the blood-brain barrier. Adv Drug Deliv Rev 1999; 36: 179-194.
15. Eytan GD, Regev R, Oren G, Assara YG. The role of passive transbilayer drug movement in multidrug resistance and its modulation. J Biol Chem 1996; 271: 12897-12902.
16. Regev R, Eytan GD. Flip-flop of doxorubicin across erythrocyte and lipid membranes. Biochem Pharmacol 1997; 54: 1151-1158.
17. Speelmans G, Staffhorst RWHM, de Kruijff B. The anionic phospholipid-mediated membrane interaction of the anti-cancer drug doxorubicin is enhanced by phosphatidylethanolamine compared to other zwitterionic phospholipids. Biochemistry 1997; 36: 8657-8662.
18. Pawagi AB, Wang J, Silverman M, Reithmeier RAF, Deber CM. Transmembrane aromatic amino acid distribution in P-glycoprotein. A functional role in broad substrate specificity. J Mol Biol 1994; 235: 554-564.
19. Sharom FJ. The P-glycoprotein efflux pump: how does it transport drugs? J Membr Biol 1997; 160: 161-175.
20. Levin VA. Relationship of octanol/water partition coefficient and molecular weight to rat brain capillary permeability. J Med Chem 1980; 23: 682-684.
21. Oldendorf WH. Lipid solubility and drug penetration of the blood brain barrier. Proc Soc Exp Biol Med 1974; 147: 813-815.
22. Barrand MA, Robertson KJ, von Weikersthal SF. Comparisons of P-glycoprotein expression in isolated rat brain microvessels and in primary cultures of endothelial cells derived from microvasculature of rat brain, epididymal fat pad and from aorta. FEBS Lett 1995; 374: 179-183.

23. Demeule M, Labelle M, Régina A, Berthelet F, Beliveau R. Isolation of endothelial cells from brain, lung, and kidney: expression of the multidrug resistance P-glycoprotein isoforms. Biochem Biophys Res Commun 2001; 281: 827-834.
24. Doran A, Obach RS, Smith BJ, Hosea NA, Becker S, et al. The impact of P-glycoprotein on the disposition of drugs targeted for indications of the central nervous system: evaluation using the *mdr1a/1b* knockout mouse model. Drug Metab Dispos 2005; 33: 165-174.
25. Jonker JW, Wagenaar E, van Deemter L, Gottschlich R, Bender HM, Dasenbrock J, Schinkel AH. Role of blood-brain barrier P-glycoprotein in limiting brain accumulation and sedative side-effects of asimadoline, a peripherally acting analgaesic drug. Br J Pharmacol 1999; 127: 43-50.
26. Tamai I, Kido Y, Yamashita J, Sai Y, Tsuji A. Blood-brain barrier transport of H_1-antagonist ebastine and its metabolite carebastine. J Drug Target 2000; 8: 383-393.
27. Kivistö KT, Zukunft J, Hofmann U, Niemi M, Rekersbrink S, Schneider S, Luippold G, Schwab M, Eichelbaum M, Fromm MF. Characterisation of cerivastatin as a P-glycoprotein substrate: studies in P-glycoprotein-expressing cell monolayers and *mdr1a/b* knock-out mice. Naunyn Schmiedebergs Arch Pharmakol 2004; 370: 124-130.
28. Yamazaki M, Neway WE, Ohe T, Chen I, Rowe JF, Hochman JH, Chiba M, Lin JL. In vitro substrate identification studies for P-glycoprotein-mediated transport: species difference and predictability of in vivo results. J Pharmacol Exp Ther 2001; 296: 723-735.
29. Ejsing TB, Hasselstrøm J, Linnet K. The influence of P-glycoprotein on cerebral and hepatic concentrations of nortriptyline and its metabolites. Drug Metab Drug Interact 2006; 21: 139-162.
30. Schuetz EG, Umbenhauer DR, Yasuda K, Brimer C, Nguyen L, Relling MV, Schuetz JD, Schinkel AH. Altered expression of hepatic cytochromes P-450 in mice deficient in one or more *mdr1* genes. Mol Pharmacol 2000; 57: 188-197.
31. Kemper EM, Verheij M, Boogerd W, Beijnen JH, Van Tellingen O. Improved penetration of docetaxel into the brain by co-administration of inhibitors of P-glycoprotein. Eur J Cancer 2004; 40: 1269-1274.
32. Tahara H, Kusuhara H, Fuse E, Sugiyama Y. P-glycoprotein plays a major role in the efflux of fexofenadine in the small intestine and blood-brain barrier, but only a limited role in its biliary excretion. Drug Metab Dispos 2005; 33: 963-968.
33. Kim RB, Fromm MF, Wandel C, Leake B, Wood AJJ, Roden DM, Wilkinson GR. The drug transporter P-glycoprotein limits oral absorption and brain entry of HIV-1 protease inhibitors. J Clin Invest 1998: 101: 289-294.
34. Wang JS, Taylor R, Ruan Y, Donovan JL, Markowitz JS, DeVane CL. Olanzapine penetration into brain is greater in transgenic *Abcb1a* P-glycoprotein deficient mice than FVB1 (wild-type) animals. Neuropsychopharmacology 2004; 29: 551-557.
35. Karssen AM, Meijer OC, van der Sandt ICJ, de Boer AG, de Lange ECM, de Kloet ER. The role of the efflux transporter P-glycoprotein in brain penetration of prednisolone. J Endocrinol 2002; 175: 251-260.

36. Wang JS, Ruan Y, Taylor RM, Donovan JL, Markowitz JS, DeVane CL. Brain penetration of methadone (*R*)- and (*S*)-enantiomers is greatly increased by P-glycoprotein deficiency in the blood-brain barrier of *Abcb1a* gene knockout mice. Psychopharmacology (Berl) 2004; 173: 132-138.
37. Wang JS, Ruan Y, Taylor RM, Donovan JL, Markowitz JS, DeVane CL. The brain entry of risperidone and 9-hydroxyrisperidone is greatly limited by P-glycoprotein. Int J Neuropsychopharmacol 2004; 7: 415-419.
38. Yokogawa K, Takahashi M, Tamai I, Kanishi H, Nomura M, Moritani S, Miyamoto K, Tsuji A. Modulation of *mdr1a* and *CYP3A* gene expression in the intestine and liver as possible cause of changes in the cyclosporin A disposition kinetics by dexamethasone. Biochem Pharmacol 2002; 63: 777-783.
39. van Asperen J, Schinkel AH, Beijnen JH, Nooijen WJ, Borst P, van Tellingen O. Altered pharmacokinetics of vinblastine in Mdr1a P-glycoprotein-deficient mice. J Natl Cancer Inst 1996; 88: 994-999.
40. De Lange ECM, Marchand S, Van den Berg DJ, van der Sandt ICJ, de Boer AG, Delon A, Bouquet S, Couet W. In vitro and in vivo investigation on fluoroquinolones; effects of the P-glycoprotein efflux transporter on brain distribution of sparfloxacin. Eur J Pharm Sci 2000; 12: 85-93.
41. Marzolini C, Paus E, Buclin T, Kim RB. Polymorphisms in human *MDR1* (P-glycoprotein): recent advances and clinical relevance. Clin Pharmacol Ther 2004; 75: 13-33.
42. Dai H, Marbach P, Lemaire M, Hayes M, Elmquist WF. Distribution of STI-571 to the brain is limited by P-glycoprotein-mediated efflux. J Pharmacol Exp Ther 2003; 304: 1085-1092.
43. Hoffmeyer S, Burk O, von Rischter O, Arnold HP, Brockmöller J, Johne A, Cascorbi I, Gerloff T, Roots I, Eichelbaum M, Brinkmann U. Functional polymophisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. Proc Natl Acad Sci USA 2000; 97: 3473-3478.
44. Nakamura T, Sakaeda T, Horinouchi M, Tamura T, Aoyama N, Shirakawa T, Matsuo M, Kasuga M, Okumura K. Effect of the mutation (C3435T) at exon 26 of the *MDR1* gene on expression level of MDR1 messenger ribonucleic acid in duodenal enterocytes of healthy Japanese subjects. Clin Pharmacol Ther 2002; 71: 297-303.
45. Roberts RL, Joyce PR, Mulder RT, Begg EJ, Kennedy MA. A common P-glycoprotein polymorphism is associated with nortriptyline-induced postural hypotension in patients treated for major depression. Pharmacogenomics J 2002; 2: 191-196.
46. De Luca V, Mundo E, Trakalo J, Wong GWH, Kennedy JL. Investigation of polymorphism in the *MDR1* gene and antidepressant-induced mania. Pharmacogenomics J 2003; 3: 297-299.
47. Uhr M, Steckler T, Yassouridis A, Holsboer F. Differential enhancement of antidepressant penetration into the brain in mice with *abcb1ab* (*mdr1ab*) P-glycoprotein gene disruption. Neuropsychopharmacology 2000; 22: 380-387.
48. Eichelbaum M, Fromm MF, Schwab M. Clinical aspects of the *MDR1* (*ABCB1*) gene polymorphism. Ther Drug Monit 2004; 26: 180-185.

49. Hugnet C, Cadore JL, Buronfosse F, Pineau X, Mathet T, Berny PJ. Loperamide poisoning in the dog. Vet Hum Toxicol 1996; 38: 31-33.
50. Sakaeda T, Nakamura T, Okumura K. Pharmacogenetics of *MDR1* and its impact on the pharmacokinetics and pharmacodynamics of drugs. Pharmacogenomics 2003; 4: 397-410.
51. Kurata Y, Ieri I, Kimura M, Morita T, Irie S, Urae A, Ohdo S, Ohtani H, Sawada Y, Higuchi S, Otsubo K. Role of human *MDR1* gene polymorphism in bioavailability and interaction of digoxin, a substrate of P-glycoprotein. Clin Pharmacol Ther 2002; 72: 209-219.
52. Sartor LL, Bentjen SA, Trepanier L, Mealey KL. Loperamide toxicity in a collie with the *mdr1* mutation associated with ivermectin sensitivity. J Vet Intern Med 2004; 18: 117-118.
53. Mealey KL, Bentjen SA, Gay JM, Cantor GH. Ivermectin sensitivity in collies is associated with a deletion mutation of the *mdr1* gene. Pharmacogenetics 2004; 11: 727-733.