Published in final edited form as:

Curr Drug Metab. 2011 October ; 12(8): 742–749.

Blood-Brain Barrier P450 Enzymes and Multidrug Transporters in Drug Resistance: A Synergistic Role in Neurological Diseases

Chaitali Ghosh¹, Vikram Puvanna¹, Jorge Gonzalez-Martinez^{2,3}, Damir Janigro^{1,2}, and Nicola Marchi^{1,*}

¹Department of Cell Biology, Cerebrovascular Research, 9500 Euclid Avenue, Cleveland Clinic, Cleveland, OH 44195

²Department of Neurosurgery, 9500 Euclid Avenue, Cleveland Clinic, Cleveland, OH 44195

³Epilepsy Center, 9500 Euclid Avenue, Cleveland Clinic, Cleveland, OH 44195

Abstract

Drug penetration into the central nervous system (CNS) is controlled by the blood-brain barrier (BBB). Even though a number of strategies to circumvent the BBB and to improve drug access have been developed, drug resistance in CNS diseases remains an unmet clinical problem. We here review the mechanisms by which a healthy or pathological BBB influences drug distribution in the brain, with emphasis on the role of P450 metabolic enzymes and multi-drug transporter (MDT) proteins. In addition to the classic hepatic and gut biotransformation pathways, CNS expression of P450 enzymes may bear pharmacokinetic and pharmacodynamic significance exerting a metabolic activity and transforming parent drugs into specific products. We propose these mechanisms to play a major role in CNS drug resistant pathologies including refractory forms of epilepsy.

Changes in the cerebrovascular hemodynamic conditions can affect expression of P450 enzymes and MDT proteins. This should be taken into account when developing *in vitro* experimental approaches to reproduce the physiological or pathological properties of the BBB. Finally, a link between P450 and MDT expression in the diseased brain and cell survival is discussed.

Keywords

Blood-brain barrier; drug resistant epilepsy; multidrug transporters proteins (MDT); P450 enzymes

THE BLOOD-BRAIN BARRIER: A BRIEF OVERVIEW

The brain microvascular endothelial cells that constitute the blood-brain barrier (BBB) are responsible for the passage of xenobiotics and nutrients from the blood into the brain parenchyma, and *vice versa*. At the cellular level, the BBB consists of endothelial cells

* Addresses correspondence to this author at the Molecular Medicine Cerebrovascular Research Cleveland Clinic Foundation NB-20 9500 Euclid Ave Cleveland, OH 44195; Tel: 216-4450562; Fax: 216-4451466; ; Email: marchin@ccf.org.

CONFLICT OF INTEREST

Drs Janigro and Marchi own shares of Flocel Inc., Cleveland, Ohio, USA. Other authors have no conflict of interest to disclose.

lining the brain microvessels together with the closely associated astrocytic end-feet processes [1-5]. The capillary endothelium is characterized by the presence of tight junctions, lack of fenestrations, and minimal presence of pinocytotic vesicles.

Endothelial tight junctions restrict the penetration of the majority of molecules and cells circulating in the blood stream. When driven by a concentration gradient and in absence of active transport only low molecular weight lipophilic compounds can diffuse into the brain [2]. Overall, the BBB function depends on a combination of physical (tight junctions), transport (specific mechanisms mediating solute flux), and metabolic (enzymes) mechanisms which, in concert, strictly regulate the passage of molecules across the brain endothelium [3]. The BBB is anatomically and functionally coupled with brain parenchymal cells, such as neurons. The distance between a BBB capillary and a given neuron is of few micrometers while the overall surface of exchange between the BBB and the brain parenchyma reaches 20 m² in the adult human brain [2]. The latter underscores the extent of the vascular-to-parenchyma communication (*e.g.*, neuro-vascular unit). The BBB is present in most of the brain regions, with the exception of circumventricular organs including area postrema, median eminence, neurohypophysis, pineal gland, subfornical organs, and lamina terminalis. Blood vessels in these areas have fenestrations permitting diffusion of blood-borne molecules across the vessel wall [6]. BBB functionality changes significantly under pathological conditions [4].

DRUG RESISTANCE IN CNS DISEASES

Drug resistance in brain diseases is an unsolved clinical problem. For example, drug resistant epilepsy affects approximately 20-25% of epileptics. According to the recent consensus of the International League Against Epilepsy (ILAE), “drug resistant epilepsy is defined with the failure of adequate trials of two appropriately chosen antiepileptic drugs, whether as monotherapies or in combination, to achieve sustained seizure freedom” [7]. In the past two decades, new antiepileptic drugs (AEDs) have been developed but no major reduction in the percentage of drug-resistant patients has been achieved [8,9]. The complexity of the drug resistant epileptic phenotype reflects the nature of the pathophysiological process, its possible evolution over time and individual sensitivity to drugs. In order to explain the mechanisms underlying the drug resistant condition, a *pharmacokinetic* and a *pharmacodynamic* hypothesis were proposed more than a decade ago. AED therapeutic failure may thus be due to a modification of neuronal targets (pharmacodynamic hypothesis) [10-13] or to inadequate AED brain levels (pharmacokinetic hypothesis). Overexpression of multidrug transporter (MDTs) proteins at the BBB was considered to be a mechanism responsible for the possible AED brain misdistribution [11,14-18]: overexpression of multi-drug transporters at the BBB luminal side *de facto* impedes drugs penetration into the brain parenchyma [17,19-21].

NEW VISTAS ON DRUG BIOTRANSFORMATION: BRAIN P450 ENZYMES

Until recently, most of the studies linking BBB to drug resistant epilepsy have focused on transport barrier mechanisms [11,14-22]. Transcript levels of P450 enzymes known to be responsible for the metabolism of several AEDs (CYP3A4, CYP2C9, CYP2C19,

CYP2A6 and CYP2E1) were demonstrated to be elevated in brain endothelial cells isolated from temporal lobe resections of drug resistant epileptic subjects compared to commercially available, “control” human brain endothelium [23,24]. Cytochrome P450 belongs to a super-family of enzymes (abbreviated as CYP) located in the endoplasmic reticulum or within mitochondrial membranes and are responsible for the metabolism of endogenous compounds and xenobiotics. CYP enzymes are expressed in hepatocytes where they are responsible for the metabolism of xenobiotics [25,26]. However, recent data has challenged this orthodoxy and a new, perhaps significant, role of CYPs in the brain is emerging [23,24,27,28]. Evidences support the presence of CYPs in the human and rodent brain [29-31]: brain expression of CYP1A1, 1B1, Phase II epoxide hydrolase and UDP-glucuronosyltransferase was confirmed using rodent models and human brain tissue. Finally, the expression of CYPs enzymes was demonstrated in an immortalized brain endothelial cell line [32,33]. Table 1 summarizes the patterns of expression of Phase I and II metabolic enzymes in the brain. Recent data demonstrate that cytochrome CYP3A4 is functionally expressed at the drug resistant, epileptic BBB [23,24]. CYP3A4 oxidizes a large group of xenobiotics, including first generation AEDs [31,34,35]. CYP3A isoforms in humans include 3A3, 3A4, 3A5 and 3A7, sharing at least 85% amino acid sequence homology [36].

Taken together, experimental evidence points to a mechanism regulating the brain bioavailability of AEDs similar to the one used by other organ systems (*e.g.*, gut and liver). In other words, P450 enzymes and MDT proteins expressed at the BBB, or other brain cells, could affect the pattern of drug brain biotransformation and efficacy. This also demonstrates that pharmacokinetic and pharmacodynamic mechanisms can act in concert as a consequence of the pathologic expression of a single molecular entity. A schematic representation of these hypotheses is shown in Fig. 1. P450 enzymes and MDT protein could synergistically contribute to the local brain biotransformation of drugs, thus decreasing the amount of parent drug (by metabolic transformation or by active extrusion) reaching the neuronal target (Fig. 1D).

BRAIN, PERIPHERAL P450 AND DRUG METABOLISM: FOCUS ON AEDS

AEDs are metabolized by phase I and II enzymes. In particular, P450 phase I enzymes (*e.g.*, CYP3A4/5 and CYP2C9/19) are responsible for the hepatic metabolism of first generation AEDs and some second generation ones [26,37-40]. Phase II enzymes such as glutathione-S-transferase (GSTs), sulfotransferase and UDP-glucuronosyl-transferase (UGTs) are responsible for the metabolic clearance of both first and second generation AEDs [41].

Generally, first generation AEDs differ substantially from second generation AEDs in terms of metabolic transformation pathways (Phase I and II) and drug-to-drug interactions [9,37,42,43]. For example, carbamazepine (first generation AED) is a substrate and an inducer of hepatic CYP3A4 [26,38-40,44] while phenytoin serum levels are determined by CYP2C9 and CYP2C19 [38,44,45]. The metabolism of carbamazepine is decreased by the co-administration of valproic acid, leading to an increased risk of carbamazepine toxicity [26,39,40]. On the other hand, oxcarbazepine and topiramate (second generation AEDs) are considered weak CYP2C19 and CYP3A4/5 substrates [46] while lamotrigine and levetiracetam are not CYP substrates [37,47]. Levetiracetam lacks of liver Phase I P450

metabolism and does not induce P450 enzymes *in vitro* [37,47]. Other important CYP inducers are barbiturates [48] such as phenobarbital which is known to increase the levels of CYP2B6 [49]. Both first and second generation AED undergo Phase II metabolism. The exact pattern of expression and the functional relevance of these enzymes in the drug resistant brain remain to be elucidated. However, based on available data, one could envision a scenario where P450 enzymes, over-expressed at the pathological BBB (e.g., drug resistant epilepsy) or in other brain cells (e.g., neurons), could locally metabolize AEDs. A mechanism involving both CYPs and MDT proteins could shape the pattern of AEDs brain bioavailability (Fig. 1). In addition, Phase II biotransformation could further shape the levels of brain AEDs. The drug resistant brain could represent a second line of drug biotransformation, significantly changing the pharmacokinetic and pharmacodynamic properties of brain drugs, such as AEDs.

An obvious confounding factor is represented by drug-to-drug interactions, especially in patients receiving multi-therapies or undergoing several drug rotations [9,50-56]. A recent review article examined the up-to-date information on drug interactions in epilepsy therapy. Besides systemic drug-to-drug interactions, the pharmacokinetic interactions that take place in the brain are currently very difficult to measure [37,47,57-59]. Moreover, the possibility exists that brain CYPs may be mutated and operate differently compared to the hepatic ones [23,24]. The latter represents an interesting, yet unstudied, hypothesis.

MDT PROTEINS EXPRESSION AT THE EPILEPTIC BBB: FROM PAST TO PRESENT INTERPRETATIONS

Overexpression of MDT proteins (MDR1, MRP1, MRP2 and BCRP [11,15-17,19,22,60]; see Table 1) at the drug-resistant epileptic BBB is likely to diminish the amount of AEDs reaching the neuronal target. Evidence connecting MDT proteins overexpression with pharmacoresistance of AEDs are strongest for MDR1 as suggested by *in vitro* and *in vivo* studies [61]. Recently, a new twist to the MDR1 tale has been suggested [62]. It was proposed that MDR1 over-expression could be related to a pro-inflammatory cascade occurring in the seizing brain. In particular, using rodent models, recent studies have demonstrated a link between cyclooxygenase-2 (COX-2), prostaglandin E2, NF-kB and MDR1 brain expression [63]. A non-selective COX inhibitor, reduced MDR1 expression and seizures in an experimental model [61]. It remains to be demonstrated how this signaling cascade could be targeted in patients to reduce pharmacoresistance.

P450, MDT AND BRAIN CELL SURVIVAL

MDT protein transcription (e.g., MDR1) is controlled by pregnane X receptors (PXR/SXR), a nuclear receptor family also involved in the regulation of P450 enzymes. PXR was found in isolated brain capillaries and linked to MDR1 expression [61]. CYP3A4 and MDR1 are co-expressed at the BBB and neurons of drug resistant epileptic subjects [24]. The PXR/SXR mechanism, previously considered to be exclusively hepatic, provides the molecular basis for the presence of P450 enzymes and drug transporters at the BBB. It is reasonable to hypothesize that CYP3A4 and MDR1 constitute machinery responsible for xenobiotics/poison detoxification in the drug resistant epileptic brain. For example, both

MDR1 and CYP3A4 have a protective role [24]. Since CYP3A4 and MDR1 are both under the transcriptional control of PXR, one may speculate that PXR is a viable target to modulate the expression of multiple drug resistance mechanisms. Finally, CYP3A4 expressed by drug resistant epileptic neurons may confer, together with MDR1, a protective mechanism shielding neurons from hostile stressors such as seizure by-products [17,24,64].

RELEVANCE OF P450 AND MDT TO OTHER CNS DISORDERS

Brain P450 enzymes and MDT proteins were suggested to play a role in neurodegenerative disorders including Alzheimer's (AD) and Parkinson's diseases (PD). Increase in brain cholesterol levels during the progression of AD is reported [65] and cholesterol-lowering drugs (e.g., statins) are used for potential treatments for AD [66,67]. Interestingly, CYP46 and CYP27 are involved in the brain metabolism of cholesterol. CYP46A1 is the main enzyme responsible for cholesterol elimination by neurons. Cholesterol 24-hydroxylase or CYP46 may constitute a potential target for the treatment of neurodegenerative diseases, if upregulation of CYP46 prevents the formation of β -amyloid.

In a model of Parkinson's disease (1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine, MPTP) a protective role of CYP2D6 was suggested. CYP2D6 catalyzes MPTP N-demethylation [68] thereby decreasing its toxic effect to the substantia nigra [69]. Abnormal levels of serotonin and other monoamine neurotransmitters (such as norepinephrine and dopamine) trigger depression and are implicated with obsessive-compulsive, phobias and anxiety disorders. CYP2D6 metabolizes neurotransmitters such as catecholamine. It has been observed that CYP2D6 and 2C19 polymorphisms are associated with the length of illness in psychiatric patients [70]. In all these CNS disorders, the relative contribution of brain vs. liver P450 remains unknown.

Decreased expression or transport function for BBB MDR1 was reported for AD [71], Creutzfeldt-Jakob disease [72], PD [73], HIV infection [74] and aging. In addition, reports suggest that glial cells also contribute to an altered distribution of therapeutic compounds in the CNS by acting as a 'secondary barrier' contributing to the drug resistance phenomenon [74-76]. It has been proposed that a cascade of neurovascular events alters BBB function and fuels disease progression in AD [77]. One element of this hypothesis is the reduction of β -amyloid efflux from the brain and the subsequent increase of brain accumulation of this pathological protein [77]. MDR1 and BCRP have been implicated as possible efflux pumps for β -amyloid itself and available data indicate that AD patients exhibit reduced expression of MDR1 [71]. Upregulation of MDR1 expression slowing β -amyloid deposition may also delay disease progression. Other evidence obtained using a transgenic AD mouse model demonstrated that brain capillary MDR1 expression is reduced; treatment with a PXR ligand upregulated MDR1 expression and reduced β -amyloid deposition [78].

MODELING THE DRUG RESISTANT BBB

Appropriate modeling of the human BBB and of the pharmacokinetic mechanisms of drug resistance represents a scientific Holy Grail. It is becoming increasingly evident that the *in vivo* physiological conditions must be fully mimicked when performing *in vitro*

experiments. In particular, data support the use of *in vitro* systems recapitulating the geometrical and physiological properties of the *in vivo* BBB [23,79-83]. Using a flow based *in vitro* BBB system (DIV-BBB) we have been able to reproduce metabolic and transport mechanisms of the drug resistant BBB as observed *in vivo* [23,24,84]. Exposure to laminar flow has been shown to affect brain endothelial cell differentiation, tight-junction formation, and regulation of cell-cycle leading to mitotic arrest [23,85,86]. Exposure to laminar flow significantly changes the levels of several P450 enzymes (CYP3A4, CYP 2C9, CYP 2C19, CYP 1A1, CYP 1B1, CYP 2A6, CYP 2B6, CYP 2E1, CYP 2J2) and MDT expression (MDR1, MRP5, RLIP76/RalBP-1, MRP1) in BBB human endothelial cells [23]. This is of relevance in the epileptic brain where local changes of vascular perfusion, occurring during the interictal to ictal transition, could possibly shape the expression of CYPs and MDT. The mechanisms by which the effect of flow becomes operant remain to be elucidated. Based on this evidence one could hypothesize a *dynamic* model of brain drug resistance where changes in the cerebral blood perfusion could affect the kinetics of drugs by altering transporter and enzymatic functions at the BBB.

CONCLUSIONS

P450 enzymes and MDT transporters synergistically control the pattern of AEDs brain penetration, ultimately dictating their pharmacological efficacy. Consideration is also given to other neurological disorders where a similar mechanism could play a role. The quest for new brain drugs has been hindered by the lack of appropriate models, perhaps due to the fact that multiple drug resistance is a patient-specific issue that cannot be appropriately reproduced with the models available. We underscored how the use of the proper experimental paradigms is of crucial importance to fully understand the mechanisms underlying the drug resistant condition: it is important to use models able to dissect out the players taking part to the phenomenon while also respecting *in vivo* variables.

ACKNOWLEDGEMENT

This work is supported by the Epilepsy Foundation Research Grant awarded to Dr. Nicola Marchi. Also supported by the Cleveland Clinic-Swedish Satellite Laboratory Feasibility Project, Federal Appropriation Washington State, and R21 HD057256 to DJ.

ABBREVIATIONS

BBB	Blood-brain barrier
CNS	Central Nervous System
P450	Cytochrome P450
AEDs	Antiepileptic drugs
DRE	Drug Resistant Epilepsy
PXR	Pregnane X Receptor
RLIP76 or RalBP-1	Ral-binding GTPase activating protein
MDR1	Multidrug resistance gene 1

MRP2	Multidrug resistance-associated protein 2
MRP5	Multidrug resistance-associated protein 5
BCRP	Breast cancer resistance protein
OATP	Organic anion transporting polypeptide
ILAE	International League against Epilepsy
DIV-BBB	Dynamic <i>In Vitro</i> Blood brain barrier

REFERENCES

- Abbott NJ. Cell Mol.Neurobiol. 2005; 25(1):5–23. [PubMed: 15962506]
- Abbott NJ, Ronnback L, Hansson E. Nat.Rev.Neurosci. 2006; 7(1):41–53. [PubMed: 16371949]
- Abbott NJ, Dolman DE, Patabendige AK. Curr.Drug Metab. 2008; 9(9):901–910. [PubMed: 18991587]
- Abbott NJ, Patabendige AA, Dolman DE, Yusof SR, Begley DJ. Neurobiol.Dis. 2010; 37(1):13–25. [PubMed: 19664713]
- Pardridge WM. Drug Discov.Today. 2007; 12(1-2):54–61. [PubMed: 17198973]
- Ballabh P, Braun A, Nedergaard M. Neurobiol.Dis. 2004; 16(1):1–13. [PubMed: 15207256]
- Kwan P, Arzimanoglou A, Berg AT, Brodie MJ, Allen HW, Mathern G, Moshe SL, Perucca E, Wiebe S, French J. Epilepsia. 2010; 51(6):1069–1077. [PubMed: 19889013]
- Granata T, Marchi N, Carlton E, Ghosh C, Gonzalez-Martinez J, Alexopoulos AV, Janigro D. Expert Rev.Neurother. 2009; 9(12):1791–1802. [PubMed: 19951138]
- Perucca E, French J, Bialer M. Lancet Neurol. 2007; 6(9):793–804. [PubMed: 17706563]
- Beck H. Epilepsia. 2007; 48(Suppl 1):14–18. [PubMed: 17316408]
- Loscher W, Sills GJ. Epilepsia. 2007; 48(12):2370–2372. [PubMed: 18088269]
- Remy S, Gabriel S, Urban BW, Dietrich D, Lehmann TN, Elger CE, Heinemann U, Beck H. Ann.Neurol. 2003; 53(4):469–479. [PubMed: 12666114]
- Remy S, Urban BW, Elger CE, Beck H. Eur.J.Neurosci. 2003; 17(12):2648–2658. [PubMed: 12823472]
- Dombrowski SM, Desai SY, Marroni M, Cucullo L, Goodrich K, Bingaman W, Mayberg MR, Benge L, Janigro D. Epilepsia. 2001; 42(12):1501–1506. [PubMed: 11879359]
- Loscher W, Potschka H. Nat.Rev.Neurosci. 2005; 6(8):591–602. [PubMed: 16025095]
- Loscher W, Potschka H. Prog.Neurobiol. 2005; 76(1):22–76. [PubMed: 16011870]
- Marchi N, Hallene KL, Kight KM, Cucullo L, Moddel G, Bingaman W, Dini G, Vezzani A, Janigro D. BMC Med. 2004; 2:37. [PubMed: 15473912]
- Sisodiya SM, Goldstein DB. Epilepsia. 2007; 48(12):2369–2370. [PubMed: 18088268]
- Marchi N, Gonzalez-Martinez J, Nguyen MT, Granata T, Janigro D. Clin.Pharmacol.Ther. 2010; 87(1):13–15. [PubMed: 20019694]
- Potschka H, Fedrowitz M, Loscher W. Neurosci.Lett. 2002; 327(3):173–176. [PubMed: 12113905]
- Potschka H, Baltes S, Loscher W. Epilepsy Res. 2004; 58(2-3):85–91. [PubMed: 15120740]
- Marchi N, Guiso G, Rizzi M, Pirker S, Novak K, Czech T, Baumgartner C, Janigro D, Caccia S, Vezzani A. Epilepsia. 2005; 46(10):1613–1619. [PubMed: 16190932]
- Ghosh C, Gonzalez-Martinez J, Hossain M, Cucullo L, Fazio V, Janigro D, Marchi N. Epilepsia. 2010; 51(8):1408–1417. [PubMed: 20074231]
- Ghosh C, Marchi N, Desai NK, Puvanna V, Hossain M, Gonzalez-Martinez J, Alexopoulos AV, Janigro D. Epilepsia. 2011; 52(3):562–571. [PubMed: 21294720]
- Dutheil F, Beaune P, Lorient MA. Biochimie. 2008; 90(3):426–436. [PubMed: 17997991]

26. Pearce RE, Lu W, Wang Y, Uetrecht JP, Correia MA, Leeder JS. *Drug Metab Dispos.* 2008; 36(8): 1637–1649. [PubMed: 18463198]
27. Gherzi-Egea JF, Strazielle N. *Microsc.Res.Tech.* 2001; 52(1):83–88. [PubMed: 11135451]
28. Gherzi-Egea JF, Strazielle NJ. *Drug Target.* 2002; 10(4):353–357.
29. Miksys S, Tyndale RF. *Drug Metab Rev.* 2004; 36(2):313–333. [PubMed: 15237857]
30. Tyndale RF, Li Y, Li NY, Messina E, Miksys S, Sellers EM. *Drug Metab Dispos.* 1999; 27(8):924–930. [PubMed: 10421620]
31. Woodland C, Huang TT, Gryz E, Bendayan R, Fawcett JP. *Drug Metab Rev.* 2008; 40(1):149–168. [PubMed: 18259987]
32. Dauchy S, Dutheil F, Weaver RJ, Chassoux F, Daumas-Duport C, Couraud PO, Scherrmann JM, De W,I, Decleves X. *J.Neurochem.* 2008; 107(6):1518–1528. [PubMed: 19094056]
33. Dauchy S, Miller F, Couraud PO, Weaver RJ, Weksler B, Romero IA, Scherrmann JM, De W,I, Decleves X. *Biochem.Pharmacol.* 2009; 77(5):897–909. [PubMed: 19041851]
34. Dresser GK, Spence JD, Bailey DG. *Clin.Pharmacokinet.* 2000; 38(1):41–57. [PubMed: 10668858]
35. Riffel AK, Schuenemann E, Vyhldal CA. *Mol.Pharmacol.* 2009; 76(5):1104–1114. [PubMed: 19706729]
36. Kolars JC, Lown KS, Schmiedlin-Ren P, Ghosh M, Fang C, Wrighton SA, Merion RM, Watkins PB. *Pharmacogenetics.* 1994; 4(5):247–259. [PubMed: 7894497]
37. Anderson GD. *Ther.Drug Monit.* 2008; 30(2):173–180. [PubMed: 18367977]
38. Caraco Y, Muszkat M, Wood AJ. *Pharmacogenetics.* 2001; 11(7):587–596. [PubMed: 11668218]
39. Pearce RE, Vakkalagadda GR, Leeder JS. *Drug Metab Dispos.* 2002; 30(11):1170–1179. [PubMed: 12386121]
40. Pearce RE, Uetrecht JP, Leeder JS. *Drug Metab Dispos.* 2005; 33(12):1819–1826. [PubMed: 16135660]
41. Bauer B, Hartz AM, Lucking JR, Yang X, Pollack GM, Miller DS. *J.Cereb.Blood Flow Metab.* 2008; 28(6):1222–1234. [PubMed: 18349876]
42. Egnell AC, Houston B, Boyer S. *J.Pharmacol.Exp.Ther.* 2003; 305(3):1251–1262. [PubMed: 12606595]
43. Egnell AC, Houston JB, Boyer CS. *J.Pharmacol.Exp.Ther.* 2005; 312(3):926–937. [PubMed: 15572649]
44. Mosher CM, Tai G, Rettie AE. *J.Pharmacol.Exp.Ther.* 2009; 329(3):938–944. [PubMed: 19258521]
45. Lakehal F, Wendum D, Barbu V, Becquemont L, Poupon R, Balladur P, Hannoun L, Ballet F, Beaune PH, Housset C. *Hepatology.* 1999; 30(6):1498–1506. [PubMed: 10573530]
46. Nallani SC, Glauser TA, Hariparsad N, Setchell K, Buckley DJ, Buckley AR, Desai PB. *Epilepsia.* 2003; 44(12):1521–1528. [PubMed: 14636322]
47. Anderson GD. *Neurology.* 2004; 63(10 Suppl 4):S3–S8. [PubMed: 15557548]
48. Bolt HM. *Drug Metab Rev.* 2004; 36(3-4):497–509. [PubMed: 15554232]
49. Wang H, Faucette SR, Gilbert D, Jolley SL, Sueyoshi T, Negishi M, LeCluyse EL. *Drug Metab Dispos.* 2003; 31(5):620–630. [PubMed: 12695351]
50. Levy RH, Rettenmeier AW, Anderson GD, Wilensky AJ, Friel PN, Baillie TA, Acheampong A, Tor J, Guyot M, Loiseau P. *Clin.Pharmacol.Ther.* 1990; 48(3):225–235. [PubMed: 2119269]
51. Mohanraj R, Brodie M. J. *Eur.J.Neurol.* 2006; 13(3):277–282. [PubMed: 16618346]
52. Neels HM, Sierens AC, Naelaerts K, Scharpe SL, Hatfield GM, Lambert WE. *Clin.Chem.Lab Med.* 2004; 42(11):1228–1255. [PubMed: 15576287]
53. Patsalos PN, Perucca E. *Lancet Neurol.* 2003; 2(8):473–481. [PubMed: 12878435]
54. Patsalos PN, Perucca E. *Lancet Neurol.* 2003; 2(6):347–356. [PubMed: 12849151]
55. Patsalos PN, Berry DJ, Bourgeois BF, Cloyd JC, Glauser TA, Johannessen SI, Leppik IE, Tomson T, Perucca E. *Epilepsia.* 2008; 49(7):1239–1276. [PubMed: 18397299]
56. Perucca E. *Br.J.Clin.Pharmacol.* 2006; 61(3):246–255. [PubMed: 16487217]
57. Anderson GD. *Ann.Pharmacother.* 1998; 32(5):554–563. [PubMed: 9606477]
58. Anderson GD. *Epilepsia.* 2002; 43(Suppl 3):53–59. [PubMed: 12060006]

59. Yap KY, Chui WK, Chan A. Clin.Ther. 2008; 30(8):1385–1407. [PubMed: 18803983]
60. Volk HA, Loscher W. Brain. 2005; 128(Pt 6):1358–1368. [PubMed: 15716304]
61. Bauer B, Hartz AM, Fricker G, Miller DS. Mol.Pharmacol. 2004; 66(3):413–419. [PubMed: 15322232]
62. Miller DS. Trends Pharmacol.Sci. 2010; 31(6):246–254. [PubMed: 20417575]
63. Bauer B, Hartz AM, Pekcec A, Toellner K, Miller DS, Potschka H. Mol.Pharmacol. 2008; 73(5): 1444–1453. [PubMed: 18094072]
64. Marroni M, Agrawal ML, Kight K, Hallene KL, Hossain M, Cucullo L, Signorelli K, Namura S, Bingaman W, Janigro D. Neuroscience. 2003; 121(3):605–617. [PubMed: 14568021]
65. Cutler RG, Mattson MP. Ageing Res.Rev. 2006; 5(3):221–238. [PubMed: 16950665]
66. Jick H, Zornberg GL, Jick SS, Seshadri S, Drachman DA. Lancet. 2000; 356(9242):1627–1631. [PubMed: 11089820]
67. Wolozin B, Kellman W, Ruosseau P, Celesia GG, Siegel G. Arch.Neurol. 2000; 57(10):1439–1443. [PubMed: 11030795]
68. Gilham DE, Cairns W, Paine MJ, Modi S, Poulsom R, Roberts GC, Wolf CR. Xenobiotica. 1997; 27(1):111–125. [PubMed: 9041683]
69. Matoh N, Tanaka S, Takehashi M, Banasik M, Stedeford T, Masliah E, Suzuki S, Nishimura Y, Ueda K. Gene Expr. 2003; 11(3-4):117–124. [PubMed: 14686785]
70. Kropp S, Lichtinghagen R, Winterstein K, Schlimme J, Schneider U. Clin.Lab. 2006; 52(5-6):237–240. [PubMed: 16812949]
71. Vogelgesang S, Cascorbi I, Schroeder E, Pahnke J, Kroemer HK, Siegmund W, Kunert-Keil C, Walker LC, Warzok RW. Pharmacogenetics. 2002; 12(7):535–541. [PubMed: 12360104]
72. Vogelgesang S, Glatzel M, Walker LC, Kroemer HK, Aguzzi A, Warzok RW. Acta Neuropathol. 2006; 111(5):436–443. [PubMed: 16523342]
73. Vautier S, Fernandez C. Expert Opin.Drug Metab Toxicol. 2009; 5(11):1349–1358. [PubMed: 19663741]
74. Langford D, Grigorian A, Hurford R, Adame A, Ellis RJ, Hansen L, Masliah E. J.Neuropathol.Exp.Neurol. 2004; 63(10):1038–1047. [PubMed: 15535131]
75. Perloff MD, von Moltke LL, Fahey JM, Greenblatt DJ. J.Pharm.Pharmacol. 2007; 59(7):947–953. [PubMed: 17637189]
76. Ronaldson PT, Persidsky Y, Bendayan R. Glia. 2008; 56(16):1711–1735. [PubMed: 18649402]
77. Zlokovic BV. Neuron. 2008; 57(2):178–201. [PubMed: 18215617]
78. Hartz AM, Miller DS, Bauer B. Mol.Pharmacol. 2010; 77(5):715–723. [PubMed: 20101004]
79. Janigro D, Leaman SM, Stanness KA. Pharm.Sci.Technolo.Today. 1999; 2(1):7–12. [PubMed: 10234198]
80. McAllister MS, Krizanac-Bengez L, Macchia F, Naftalin RJ, Pedley KC, Mayberg MR, Marroni M, Leaman S, Stanness KA, Janigro D. Brain Res. 2001; 904(1):20–30. [PubMed: 11516408]
81. Stanness KA, Guatteo E, Janigro D. Neurotoxicology. 1996; 17(2):481–496. [PubMed: 8856743]
82. Stanness KA, Westrum LE, Fornaciari E, Mascagni P, Nelson JA, Stenglein SG, Myers T, Janigro D. Brain Res. 1997; 771(2):329–342. [PubMed: 9401753]
83. Stanness KA, Neumaier JF, Sexton TJ, Grant GA, Emmi A, Maris DO, Janigro D. Neuroreport. 1999; 10(18):3725–3731. [PubMed: 10716199]
84. Cucullo L, Hossain M, Rapp E, Manders T, Marchi N, Janigro D. Epilepsia. 2007; 48(3):505–516. [PubMed: 17326793]
85. Arisaka T, Mitsumata M, Kawasumi M, Tohjima T, Hirose S, Yoshida Y. Ann.N.Y Acad.Sci. 1995; 748:543–554. [PubMed: 7695202]
86. Desai SY, Marroni M, Cucullo L, Krizanac-Bengez L, Mayberg MR, Hossain MT, Grant GG, Janigro D. Endothelium. 2002; 9(2):89–102. [PubMed: 12200960]
87. Delaporte E, Renton KW. Life Sci. 1997; 60(10):787–796. [PubMed: 9064483]
88. Meyer RP, Podvinec M, Meyer UA. Mol.Pharmacol. 2002; 62(5):1061–1067. [PubMed: 12391268]
89. Iba MM, Storch A, Ghosal A, Bennett S, Reuhl KR, Lowndes HE. Arch.Toxicol. 2003; 77(10): 547–554. [PubMed: 14574441]

90. Schilter B, Omiecinski CJ. *Mol.Pharmacol.* 1993; 44(5):990–996. [PubMed: 8246922]
91. Granberg L, Ostergren A, Brandt I, Brittebo EB. *Drug Metab Dispos.* 2003; 31(3):259–265. [PubMed: 12584151]
92. Thuerl C, Otten U, Knoth R, Meyer RP, Volk B. *Brain Res.* 1997; 762(1-2):47–55. [PubMed: 9262157]
93. Knupfer H, Knupfer MM, Hotfilder M, Preiss R. *Oncol.Res.* 1999; 11(11-12):523–528. [PubMed: 10905564]
94. Harder DR, Zhang C, Gebremedhin D. *News Physiol Sci.* 2002; 17:27–31. [PubMed: 11821533]
95. Meyer RP, Knoth R, Schiltz E, Volk B. *Exp.Neurol.* 2001; 167(2):376–384. [PubMed: 11161626]
96. Hedlund E, Wyss A, Kainu T, Backlund M, Kohler C, Pelto-Huikko M, Gustafsson JA, Warner M. *Mol.Pharmacol.* 1996; 50(2):342–350. [PubMed: 8700142]
97. Chinta SJ, Pai HV, Upadhy SC, Boyd MR, Ravindranath V. *Brain Res.Mol.Brain Res.* 2002; 103(1-2):49–61. [PubMed: 12106691]
98. McFayden MC, Melvin WT, Murray GI. *Biochem.Pharmacol.* 1998; 55(6):825–830. [PubMed: 9586955]
99. Siegle I, Fritz P, Eckhardt K, Zanger UM, Eichelbaum M. *Pharmacogenetics.* 2001; 11(3):237–245. [PubMed: 11337939]
100. Choudhary D, Jansson I, Stoilov I, Sarfarazi M, Schenkman JB. *Arch.Biochem.Biophys.* 2005; 436(1):50–61. [PubMed: 15752708]
101. Miksys S, Lerman C, Shields PG, Mash DC, Tyndale RF. *Neuropharmacology.* 2003; 45(1):122–132. [PubMed: 12814665]
102. Upadhy SC, Tirumalai PS, Boyd MR, Mori T, Ravindranath V. *Arch.Biochem.Biophys.* 2000; 373(1):23–34. [PubMed: 10620320]
103. Nef P, Heldman J, Lazard D, Margalit T, Jaye M, Hanukoglu I, Lancet D. *J.Biol.Chem.* 1989; 264(12):6780–6785. [PubMed: 2708343]
104. Qu W, Bradbury JA, Tsao CC, Maronpot R, Harry GJ, Parker CE, Davis LS, Breyer MD, Waalkes MP, Falck JR, Chen J, Rosenberg RL, Zeldin DC. *J.Biol.Chem.* 2001; 276(27):25467–25479. [PubMed: 11328810]
105. Voirol P, Jonzier-Perey M, Porchet F, Reymond MJ, Janzer RC, Bouras C, Strobel HW, Kosel M, Eap CB, Baumann P. *Brain Res.* 2000; 855(2):235–243. [PubMed: 10677595]
106. Wang H, Kawashima H, Strobel HW. *Biochem.Biophys.Res.Commun.* 1996; 221(1):157–162. [PubMed: 8660328]
107. Hagemeyer CE, Rosenbrock H, Ditter M, Knoth R, Volk B. *Neuroscience.* 2003; 117(3):521–529. [PubMed: 12617959]
108. Stromstedt M, Warner M, Gustafsson JA. *J.Neurochem.* 1994; 63(2):671–676. [PubMed: 8035191]
109. Stanley LA, Copp AJ, Pope J, Rolls S, Smelt V, Perry VH, Sim E. *Teratology.* 1998; 58(5):174–182. [PubMed: 9839355]
110. Belanger G, Barbier O, Hum DW, Belanger A. *Eur.J.Biochem.* 1999; 260(3):701–708. [PubMed: 10102998]
111. de Leon J. *Int.J.Neuropsychopharmacol.* 2003; 6(1):57–72. [PubMed: 12899737]
112. Yu XQ, Xue CC, Wang G, Zhou SF. *Curr.Drug Metab.* 2007; 8(8):787–802. [PubMed: 18220559]
113. Zhou SF, Wang LL, Di YM, Xue CC, Duan W, Li CG, Li Y. *Curr.Med.Chem.* 2008; 15(20):1981–2039. [PubMed: 18691054]
114. Zhou SF. *Xenobiotica.* 2008; 38(7-8):802–832. [PubMed: 18668431]
115. Franke RM, Gardner ER, Sparreboom A. *Curr.Pharm.Des.* 2010; 16(2):220–230. [PubMed: 19835554]
116. Urquhart BL, Kim RB. *Eur.J.Clin.Pharmacol.* 2009; 65(11):1063–1070. [PubMed: 19727692]
117. Hagenbuch B, Meier PJ. *Biochim.Biophys.Acta.* 2003; 1609(1):1–18. [PubMed: 12507753]
118. Tirona RG, Kim RB. *Adv.Drug Deliv.Rev.* 2002; 54(10):1343–1352. [PubMed: 12406649]
119. Awasthi S, Hallene KL, Fazio V, Singhal SS, Cucullo L, Awasthi YC, Dini G, Janigro D. *BMC Neurosci.* 2005; 6:61. [PubMed: 16188027]

120. Janigro D, Awasthi S, Awasthi YC, Sharma R, Yadav S, Singhal SS, Hallene K. *Epilepsia*. 2007; 48(6):1218–1219. [PubMed: 17553125]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Fig. (1). Schematic representation of the changes associated with the over-expression of P450 enzymes and MDT proteins at the BBB. **(A-B)** After administration, a given drug (e.g., AED, *triangles*) will undergo, or not, a first passage metabolic biotransformation (*circles*) in the liver. Through the systematic circulation, a given AED will reach the BBB and then, under non pathological conditions, **(C)** penetrate into the brain as predicted by its biophysical properties. Conversely, over-expression of CYPs and MDTs at the *diseased* BBB could result in a significantly decreased amount of drug passing through the BBB endothelium **(D)**. In particular, one quote of the parent drug will be pumped back in the vascular bed while another amount will be metabolized (*circles*) intracellularly before being released into the brain.

Table 1

Expression of Metabolizing Enzymes and Transporters in Brain

Gene Products	Species	Brain Region	Cell Type	Expression Characteristics	References
Metabolizing Enzymes Phase I					
CYP1A1	Mouse, Rat, Human	Multiple brain regions	Astrocytes, Neurons	Protein, mRNA	[87,88]
CYP1A2	Rat, Human	Cortex, Cerebellum, Brain stem, Thalamus, Hippocampus, Striatum,	Astrocytes, Neurons	Protein, mRNA	[25,89,90]
CYP1B1	Mouse	Blood-brain barrier	BBB endothelial cells	Protein	[32,33,91]
CYP2B6	Human, Rat	Cortex, Cerebellum, Hippocampus	Astrocytes, Neurons	Protein	[29]
CYP2B10	Mouse	Hippocampus	Neurons	Protein	[92]
CYP2C9	Human			mRNA	[93]
CYP2C11	Rat	Multiple brain regions	Astrocytes		[94]
CYP2C19	Human	Multiple brain region		mRNA	[37]
CYP2C29	Mouse	Blood-brain barrier	Astrocytes	Protein	[95]
CYP2D1	Rat		Neurons		[30]
CYP2D4	Rat	Substantia nigra, olfactory bulb, cerebellum	Neurons		[96]
CYP2D6	Human	Multiple regions, especially hippocampus, cortex, cerebellum	Neurons	mRNA, protein	[97-99]
CYP2E1	Rat, Human	Cortex, cerebellum, basal ganglia, hippocampus, medulla oblongata, pons	Neurons	mRNA, protein	[32,33,98,100-102]
CYP2G	Rat	Olfactory bulb	Neuro- epithelium		[103]
CYP2J2	Human	Cortex, Microvessels	Blood-brain barrier micro-vessels	mRNA	[32,33]
CYP2J9	Mouse	Cerebellum, hippocampus, cerebral cortex, brain stem	Neurons	mRNA	[104]
CYP3A4	Human, Rat	Cortex, frontal lobe, thalamus	Blood-brain barrier endothelial cells, neurons	mRNA, protein	[23,24,31,93,98,105]
CYP3A9	Rat	Cerebellum	Neurons	mRNA	[106]
CYP3A11	Mouse	Hypothalamus, hippocampus, olfactory bulb, cerebellum	Neurons, Microcapillaries, Astrocytes	Protein	[31,107]
CYP3A13	Mouse	Hippocampus, hypothalamus, Olfactory bulb	Neurons, Micro capillaries, Astrocytes	Protein	[107]
CYP4A	Rat	Cortex, Cerebellum, brain stem, hypothalamus	Neurons	mRNA	[108]
Metabolizing enzymes Phase II					
N-acetyltransferases (NATs)					
NAT1	Mouse	Cerebellum	Neurons	mRNA, protein	[109]

Gene Products	Species	Brain Region	Cell Type	Expression Characteristics	References
NAT2	mouse	Hindbrain, cerebellum	Neurons	protein	[109]
<i>Glutathione S-Transferases (GSTs)</i>					
GSTa (GSTA)	Rat	Blood-brain barrier	Isolated Brain capillaries	mRNA, protein	[41]
GST- μ (GSTBM)	Rat	Choroidal epithelium		protein	[27,28]
GST- π (GSTP)	Rat	Choroid plexus; Blood-brain barrier	Isolated Brain capillaries	protein	[27,28,41]
<i>UDP-glucuronosyltransferases (UGTs)</i>					
UGT1A	Rat	Choroid plexus		protein	[27,28]
UGT2B	Human	Various brain regions including cerebellum	Brain capillaries	mRNA, proteins	[110,111]
Transporters					
MDR1	Human; Rat	Blood-brain barrier	Astrocytes, Neurons, Brain capillaries	Protein	[17,63]
MDR3/2	Rat	Blood-brain barrier	Brain capillaries	Protein	[63]
MRP1	Human, Mouse	Blood-brain barrier	Endothelial cells	Protein	[112-114]
MRP2	Rat, human, Mouse	Blood-brain barrier	Isolated Brain capillaries	mRNA, protein	[63,112]
MRP3	Human, Mouse	Blood-brain barrier	Endothelial cells	Protein	[113,114]
MRP4	Rat, human	Blood-brain barrier	Brain capillary membranes	mRNA, protein	[41]
MRP5	Human	Blood-brain barrier	Endothelial cells	Protein	[113,114]
MRP6	Human	Blood-brain barrier	Endothelial cells	Protein	[113,114]
MRP7	Human	Blood-brain barrier	Endothelial cells	Protein	[113,114]
BCRP	Human, Mouse	Blood-brain barrier-apical luminal	Brain Endothelial cells	Protein	[115,116]
OATP-A	Human, Rat, Mouse	Blood-brain barrier	Brain Endothelial cells	Protein	[113,114,117,118]
RLIP76	Human, Rat, Mouse	Blood-brain barrier	Endothelial cells	mRNA, Proteins	[119,120]
OATP-C	Human, Rat, Mouse	Blood-brain barrier	Brain Endothelial cells	Protein	[117,118]
OATP8	Human		Certain brain cells	Protein	[117,118]
OATP-B	Human, Rat, Mouse	Blood-brain barrier	Brain Endothelial cells	Protein	[117,118]
OATP-D	Human, Rat, Mouse		Ubiquitous	Protein	[117,118]
OATP-E	Human, Rat,		Ubiquitous	Protein	[117,118]
OATP-F	Human, Rat, Mouse	Blood-brain barrier	Brain Endothelial cells	Protein	[117,118]