Targeting the Brain – Surmounting or Bypassing the Blood–Brain Barrier

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Abstract The constituents of the blood–brain barrier, including its efflux transporter system, can efficiently limit brain penetration of potential CNS therapeutics. Effective extrusion from the brain by transporters is a frequent reason for the pharmaceutical industry to exclude novel compounds from further development for CNS therapeutics. Moreover, high transporter expression levels that are present in individual patients or may be generally associated with the pathophysiology seem to be a major cause of therapeutic failure in a variety of CNS diseases including brain tumors, epilepsy, brain HIV infection, and psychiatric disorders. Increasing knowledge of the structure and function of the blood–brain barrier creates a basis for the development of strategies which aim to enhance brain uptake

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of beneficial pharmaceutical compounds. The different strategies discussed in this review aim to modulate blood–brain barrier function or to bypass constituents of the blood–brain barrier.

Keywords Blood–brain barrier · Controlled release system · Efflux transporter · Nanoparticles · P-glycoprotein · Pharmacoresistance

1 Introduction

The limited membrane permeability of cells which are constituents of the blood–tissue barrier contributes critically to protection of tissues from putatively harmful xenobiotics. On the other hand the efficient barrier can restrict the brain penetration of central nervous system (CNS) therapeutics. Therefore many promising compounds fail in CNS drug development due to limited access to the target sites in the brain. Moreover the efficacy of marketed drugs can be reduced by a restriction of brain access resulting in a low efficacy or even mere pharmacoresistance. The penetration may be limited by basal barrier function. In some diseases pathophysiology-associated changes can occur at the blood–brain barrier (BBB) which may further restrict brain access. Furthermore drugs may also affect the BBB and may further tighten the barrier. In individual patients the genetics of physiological agents which contribute to BBB structure and function can additionally affect brain access and efficacy of CNS therapeutics. When aiming to optimize brain pharmacokinetics, it is of specific interest to develop strategies to overcome, modulate, or bypass the BBB.

2 Structure and Function of the Blood–Brain Barrier

The BBB critically controls the passage of compounds from the blood to the CNS. The major component of the BBB is a monolayer of brain capillary endothelial cells. The restriction of brain penetration arises from the presence of tight junctions between adjacent endothelial cells and the relative paucity of fenestrae and pinocytotic vesicles. A basal membrane, pericytes, and astrocyte foot processes surround the brain capillary endothelial cells. The close association between brain capillary endothelial cells and surrounding astrocytes seems to be critical for the induction of barrier functions in the endothelial cell layer, including the formation of interendothelial tight junctional complexes.

Due to the BBB, circulating compounds can only gain access to the brain via lipid-mediated transport of small nonpolar molecules through the BBB by passive diffusion or less frequently by catalyzed transport (Pardridge 1999). As a

consequence there is a strong positive correlation between lipophilicity and brain access for the majority of CNS active compounds. However, uptake can be lower than predicted for compounds which are subject to extrusion from the brain by active BBB efflux transporters.

Numerous membrane transporters have been described in BBB endothelial cells which are involved in the influx or efflux of various essential substrates including electrolytes, nucleosides, amino acids, and glucose (Lee et al. 2001), as well as xenobiotics. Efflux transporters at the BBB protect the CNS tissue against changes in the environment by restricting penetration into and facilitating extrusion from brain tissue (Leslie et al. 2005). Because the transporter molecules do not distinguish between harmful xenobiotics and active pharmaceutical ingredients (API) which are used as drugs to treat CNS diseases, the brain efflux transporters can also cause undesirable effects in limiting brain access of drugs which are administered for CNS disease therapy (Loscher and Potschka 2005a). Several brain efflux transporters have been linked to a limited brain penetration of CNS active drugs which restricts drug effectiveness or may even result in mere drug resistance. More than a decade ago, P-glycoprotein (Pgp, ABCB1) was the first drug efflux transporter to be identified in the BBB (Cordon-Cardo et al. 1989; Thiebaut et al. 1987). Since then accumulating data indicate a critical role of different BBB efflux transporters in limiting the brain uptake of a variety of therapeutic agents (Loscher and Potschka 2005b).

The most relevant efflux transporters which have so far been identified at the BBB belong to the class of ABC transporters. ABC transporters comprise two transmembrane domains and two nucleotide-binding domains (Rosenberg et al. 2003), which may also be encoded by two separate polypeptides. For several ABC transporters, for example Pgp, there is more than one substrate-binding site per transporter, allowing for a broad substrate spectrum. Transport of compounds is associated with major conformational changes in the transporter molecule (Martin et al. 2001). ATP binding seems to induce a conformational change which is associated with alterations in affinity and orientation of the substrate binding site (s) such that substrate is released at the extracellular surface of the membrane (Martin et al. 2001). Subsequently, hydrolysis of ATP resets the transporter for the next cycle (Senior et al. 1995).

Depending on structural features of the encoded transporters, *ABC* genes are divided into a number of families (*ABCA, ABCB, ABCC, ABCD, ABCE, ABCF*, and *ABCG*). Due to the fact that the old nomenclature is frequently used in the publications cited in this review, this will be used throughout the chapter. The new nomenclature is given at least once with the first mention of the respective transporter.

Efflux transporters expressed at the BBB include members of the ABCB, ABCC and ABCG family: Pgp (ABCB1), members of the multidrug-resistance associated protein family (MRP/ABCC family), and breast cancer related protein (BCRP/ABCG2).

For the pharmaceutical industry, the question whether a developmental compound is a transporter substrate is of particular interest. Low affinity to BBB efflux

transporters is advantageous for the development of CNS therapeutics, which have to achieve high concentrations in the brain. In contrast, high affinity to BBB efflux transporters is advantageous for the development of drugs, which should act in the periphery in order to avoid CNS side effects.

3 The Blood–Brain Barrier as a Limiting Factor in the Treatment of CNS Diseases

As already described, the function of the BBB can critically influence drug efficacy and tolerability. Compounds may be too hydrophilic to penetrate efficaciously by diffusion, whereas transport by brain efflux transporters may function as a limiting factor for brain uptake of lipophilic compounds.

Therapeutic success in many CNS diseases including brain cancer, epilepsy, depression, schizophrenia, and HIV-associated encephalopathy is limited by poor response or complete resistance to drug treatment. Besides other mechanisms, alterations in drug uptake into the brain or into brain parenchymal target cells are considered to be an important reason for therapeutic failure (Loscher and Potschka 2005a; Thuerauf and Fromm 2006). Thereby, disease-associated or therapy-induced changes in efflux transporter expression are thought to critically affect brain pharmacokinetics of a variety of important CNS active drugs.

Many brain tumors are highly resistant to drug treatment and systemic chemotherapy often fails to improve the outcome. A key factor for therapeutic failure of systemic chemotherapy is restricted BBB penetration of potent chemotherapeutic drugs (Nies 2007). Anticancer agents were among the first drugs that were identified to be substrates of BBB efflux transporters, i.e., of Pgp as well as MRPs and BCRP. Several studies indicated that the poor efficacy of systemically administered anticancer drugs is at least partly due to the activity of BBB efflux transporters (Kemper et al. 2004).

In 30%–40% of epilepsy patients antiepileptic drug therapy fails to control seizure activity in an adequate manner. Microdialysis experiments using transporter inhibitors, experiments in knockout mice, as well as in vitro studies have indicated that several antiepileptic drugs are transported by BBB Pgp, and some are also subject to transport by MRPs (Cucullo et al. 2007; Loscher and Potschka 2005b; Marchi et al. 2005; Rizzi et al. 2002; Sills et al. 2002). As antiepileptic drugs generally penetrate well into the brain, they can only be low to medium affinity substrates. Brain penetration of antiepileptic drugs is only restricted when an overexpression of BBB efflux transporters occurs as a consequence of seizure activity. Therefore assays with sufficient sensitivity are required to determine whether antiepileptic drugs are substrates of BBB transporters. In recent years the neglect of this fact resulted in a series of inconsistent data. Thus, further research will be necessary to determine whether all clinically relevant antiepileptic drugs are substrates of BBB efflux transporters, especially of the human isoforms.

Seizure-induced overexpression of BBB efflux transporters in the epileptic brain generally renders a feasible explanation for the multidrug resistance of epilepsy based on limited access of antiepileptic drugs to their target sites. Important support for this concept came from experiments in two different models of drug-resistant epilepsy. Pgp expression in drug-resistant rats significantly exceeded that in drug-responsive rats (Potschka et al. 2004; Volk and Loscher 2005). Recent experimental studies, in which it was demonstrated that drug resistance of seizures can be overcome by transporter inhibitors, rendered further evidence for the multidrug transporter hypothesis of drug-resistant epilepsy (Brandt et al. 2006; Clinckers et al. 2005). When further developing or validating new strategies to overcome drug resistant epilepsy, it must be taken into consideration that it is considered a multifactorial problem, and thus the relative importance of efflux transporter over-expression needs to be elucidated.

Genetic deficiency of Pgp in mice resulted in enhanced brain access for several antidepressants, indicating that these are effluxed into the blood by Pgp (Grauer and Uhr 2004; Uhr and Grauer 2003; Uhr et al. 2003, 2000). Whether this active efflux transport can contribute to therapeutic failure in depression remains to be determined. Due to the lack of models for treatment-resistant psychiatric disorders, it is difficult to test the validity of this hypothesis, which therefore still remains rather speculative. First indirect support for an impact of BBB efflux transporters on therapeutic success in the treatment of psychiatric diseases came from a genetic analysis in schizophrenic patients treated with bromperidol. The MDR1 genotype showed correlation with the therapeutic response to bromperidol (Yasui-Furukori et al. 2006). Recently, Uhr et al. (2008) reported that the MDR1 genotype of depressed patients is a strong predictor for therapeutic success with several antidepressants.

In the treatment of HIV infection, the development of HIV protease inhibitors has resulted in considerable progress. However, a major limitation in their efficacy is the restricted access to the brain which leaves the brain viral reservoir unaffected. Pgp-mediated efflux has been hypothesized to contribute to the limited brain penetration rates of HIV protease inhibitors such as saquinavir, amprenavir, nelfinavir, and indinavir (Banks et al. 2006; Edwards et al. 2002; Kim et al. 1998; Washington et al. 2000). Pgp upregulation at the BBB by the HIV-Tat protein (Hayashi et al. 2005) may further reduce penetration and efficacy of the HIV protease inhibitors in long-term survivors of AIDS. In addition to Pgp, MRP1, MRP2, and MRP4 also accept HIV protease inhibitors as substrates and might therefore be involved in the limitation of their brain access.

Riluzole is the only recognized drug that increases the survival time of patients with amyotrophic lateral sclerosis. Studies in Pgp knockout mice revealed that riluzole and minocycline, a compound which can delay disease onset, are both substrates of Pgp (Milane et al. 2007), so that therapeutic efficacy may be affected by this BBB efflux transporter.

Pgp-mediated extrusion from the brain has a tremendous impact on opiate and opioid analgesic efficacy. Modulation of Pgp function significantly affected the antinociceptive effect of morphine (King et al. 2001; Letrent et al. 1999; Thompson

Table 1 CNS therapeutics as substrates of BBB efflux transporters: examples

Pharmacological group	Examples	Transporters involved
Anticancer drugs	Doxorubicin, daunorubicin, vinblastin, vincristine, paclitaxel, etoposide, topotecan	ABCB1/Pgp, ABCC transporters/MRPs, ABCG2/BCRP
Analgesics	Morphine, methadone, fentanyl	ABCB1/Pgp
HIV protease inhibitors	Amprenavir, indinavir, saquinavir	ABCB1/Pgp, ABCC transporters/MRPs
Antipsychotic agents	Olanzapine, amisulpride	ABCB1/Pgp
Antiepileptic drugs	Phenytoin, carbamazepine, oxcarbazepine, lamotrigine, phenobarbital, felbamate, valproic acid, topiramate	ABCB1/Pgp, ABCC transporters/MRPs
Antidepressants	Amitryptiline, nortryptiline, venlafaxine, paroxetine	ABCB1/Pgp

The transporters listed do not necessarily transport all compounds given, but in some cases transport only single compounds of the pharmacological group

et al. 2000). Thus, Pgp seems to be an important issue in pain control with opioid analgesics, which may influence the onset, magnitude, and duration of the analgesic response (Dagenais et al. 2004). Recently, this assumption was further substantiated as Hamabe et al. (2007) reported a negative correlation between morphine's analgesic effects in a mouse model and the individual Pgp expression rate in the cortex.

Furthermore, Pgp seems to limit the distribution of some antibacterial drugs including fluoroquinolones and erythromycin to the brain (Sasabe et al. 2004; Schinkel 1999). Brain extrusion of these antibiotics may contribute to their limited or lack of efficacy in CNS microbial infections.

To summarize, it has been demonstrated that BBB efflux transporters critically influence CNS effects of numerous APIs (Table 1) and that this influence is of clinical relevance for many of these drugs.

4 Modulation of Blood–Brain Barrier Function

Accumulating knowledge of the impact of the BBB and its efflux transporter system on response to CNS therapy stimulates efforts to develop strategies to target drugs in an optimized manner to the brain tissue (Fig. 1; Table 2). The development of imaging techniques based on positron emission tomography creates the opportunity to study Pgp-mediated transport noninvasively in individual patients and its modulation in vivo (Elsinga et al. 2004; Hendrikse and Vaalburg 2002; Langer et al. 2007; Lee et al. 2006). Further development of these diagnostic techniques will open avenues for selection of patients that may benefit from new strategies aiming to outwit or bypass the BBB.

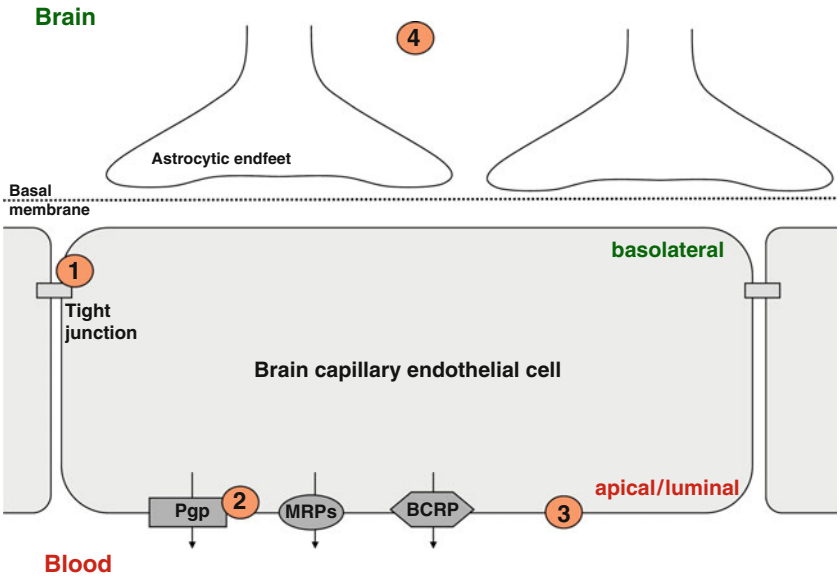

Fig. 1 Strategies to enhance brain penetration of CNS therapeutics. (1) Opening of the BBB can be achieved by disintegrating the tight junctional complex. (2) Modulation of BBB efflux transporter function or inhibition of the induction of efflux transporters results in a more specific enhancement of brain penetration rates. (3) Use of nano-sized carrier systems and drug conjugates allows bypassing of BBB efflux transporter molecules. (4) Direct intracerebral administration (e.g., by implantation of release systems) bypasses the barriers and results in much higher local drug concentrations

4.1 Opening of the BBB

BBB disruption must be transient and reversible to have any role in the delivery of APIs such as anticancer drugs to the brain. A variety of hypertonic solutions has been used to disrupt the BBB (Kroll et al. 1998). With its approval for use in patients, mannitol is most commonly used in both preclinical and clinical studies. Mannitol-mediated BBB opening has been used in combination with anticancer drugs to treat patients with metastatic or primary brain tumors. Some studies have indicated some success and minimal morbidity of the strategy. In a simplified view of the approach, mannitol is considered to result in osmotic shrinkage of endothelial cells thereby inducing tractive forces on the tight junctions which then disintegrate. However, as several structural and functional changes occur in endothelial cells in response to mannitol, the events that result in enhanced permeability may be much more complex. Recently, Farkas et al. (2005) reported that hyperosmotic mannitol induces phosphorylation of beta-catenin. Since beta-catenin is a key component of the junctional complex, its phoshorylation may be important for mannitol-induced reversible opening of the BBB.

In general, osmotic disruption of the BBB is not specific enough to exclude CNS entry of toxic xenobiotics. Furthermore BBB opening and albumin extravasation

Table 2 Strategies to enhance brain penetration

Strategy	Putative relevance for	Experimental evidence	Clinical evidence
Modulation of BBB function			
Opening/weakening of the BBB, e.g., Mannitol Bradykinin analog Alkylglycerol	Brain cancer	+	+
Inhibition of efflux transport	Brain cancer	+	–
	Epilepsy	+	–
	Focal cerebral ischemia	+	–
	Brain HIV	–	–
	Psychiatric diseases	–	–
Prevention of disease- or therapy-associated changes in BBB efflux transporter expression	Brain cancer	–	–
	Epilepsy	+	–
	Focal cerebral ischemia	–	–
Bypassing the BBB			
Nano-sized carrier systems and drug conjugates	Brain cancer	+	+
	Epilepsy	+	–
	Focal cerebral ischemia	–	–
	Brain HIV	–	–
	Psychiatric diseases	–	–
Intracerebral administration	Brain cancer	+	+
	Epilepsy	+	–

may facilitate or even cause epileptogenesis (Ivens et al. 2007; Tomkins et al. 2007; van Vliet et al. 2007). Thus, more specific strategies to target APIs to the brain without disruption of BBB integrity would be advantageous.

Administration of a bradykinin analog (RMP-7) has been suggested as an alternative approach. RMP-7 opens tight junctions by a receptor-mediated mechanism, thereby promoting delivery of the cytostatic carboplatin to glioma implanted in rat brain (Matsukado et al. 1996). However, the bradykinin analog failed to improve carboplatin efficacy in phase II and III clinical trials (Prados et al. 2003).

Further options include the intracarotid administration of alkylglycerol which affects the BBB in a more subtle way. Enhanced drug transport via the paracellular way has been described in rodents (Erdlenbruch et al. 2003a, b). To our knowledge no human data are available so far.

4.2 Inhibition of Efflux Transport

Increasing awareness of the impact of efflux transporters on successful pharmacotherapy of CNS diseases stimulates efforts to develop strategies to modulate

transporter function (Loscher and Potschka 2005a; Thuerauf and Fromm 2006). As Pgp is known to transport a large number of commonly prescribed drugs, efforts to date concentrate especially on this transporter. Mechanisms by which Pgp activity in the BBB can be modulated include direct inhibition by specific inhibitors, functional modulation, and transcriptional modulation (Bauer et al. 2005). Initially, drugs used for other indications and noted to inhibit Pgp in cell culture, such as verapamil, cyclosporin A, and quinidine, have been tested as Pgp modulators (Fox and Bates 2007). Owing to low binding affinity for Pgp, high doses of these early inhibitors were needed and excessive toxicity was observed in patients. Second generation inhibitors were developed as analogs of the initial agents. Valspodar (PSC-833), a non-immunosuppressive derivative of cyclosporin D, exemplifies the development of these agents (Fox and Bates 2007). The compound proved to be better tolerated than inhibitors of the first generation. However, valspodar inhibits CYP enzymes thereby resulting in decreased clearance and increased systemic exposure of co-administered compounds.

Whereas first and second generation Pgp inhibitors were hampered by additional pharmacodynamic effects or by additional effects on drug metabolism (Thomas and Coley 2003), the development of third generation Pgp inhibitors produced selective and more potent modulators, such as tariquidar, laniquidar, zosuquidar, and elacridar (Bates et al. 2002; Thomas and Coley 2003). The three generations of Pgp modulators comprise competitive inhibitors which are substrates by themselves, and noncompetitive inhibitors that induce changes in the conformation which affect transport efficacy.

In view of the complexity of efflux transport, an aim that suggests itself is to develop dual or multipotent inhibitors. Jekerle et al. (2006) recently reported the development of the novel inhibitor, WK-X-34, which modulates both Pgp and BCRP in experimental models. In the clinical setting, co-administration of Pgp inhibitors together with anticancer drugs in oncology has shown some efficacy (Breedveld et al. 2006), although not all studies yielded promising data. Therefore, the continued development of these agents must be awaited in order to establish the true potential of Pgp-mediated reversal of multidrug resistance in the treatment of brain cancer and other CNS diseases. In this context, it is of particular interest that a recent study reported differences in the sensitivity of Pgp located in different cells and blood–tissue barriers (Choo et al. 2006), Pgp localized in the BBB proved to be more resistant to inhibition than Pgp in other tissues (Choo et al. 2006). This resistance can be overcome by a sufficiently high dose of an inhibitor. However, whether this is safely attainable in the clinical situation remains to be determined.

Experimental studies in a rodent glioblastoma model and a rodent melanoma brain metastasis model demonstrated efficacy of the strategy (Fellner et al. 2002; Joo et al. 2008). Brain penetration and efficacy of systemically administered paclitaxel could be enhanced significantly by co-administration of the Pgp inhibitors valspodar or HM30181A (Fellner et al. 2002). Co-administration of Pgp inhibitors also improved the response to antiepileptic drugs and even helped to overcome mere resistance to antiepileptic drugs in several animal models (Brandt

et al. 2006; Clinckers et al. 2005). In these studies the combination proved to be well tolerated. In a rodent model of focal cerebral ischemia, co-administration of a third generation Pgp inhibitor was also substantiated as a promising strategy for neuroprotection (Spudich et al. 2006). Tariquidar enhanced the accumulation and the neuroprotective efficacy of the neuroprotectants FK506 and rifampicin.

In view of the experimental success, it is important to consider that any modulation of transporter function is associated with specific hazards. First, complications with a combination of a Pgp inhibitor or modulator with a CNS active drug may be related to the intended aim. An influence on pharmacokinetics of the therapeutic agent will not only affect the target tissue or target brain region. Enhanced drug concentrations in other brain regions and also in peripheral tissues may promote side effect potentiation. In accordance with this, several trials with combinations of anticancer drugs and Pgp inhibitors had to be closed ahead of schedule due to enhanced chemotherapy-related toxicity (Fox and Bates 2007).

Second, multidrug transporters such as Pgp serve a variety of physiological functions including protection from xenobiotics. Other xenobiotics taken up by the body may be more harmful in the presence of efflux transporter inhibitors due to the influence on their distribution. Furthermore Pgp and MRPs may protect brain parenchymal cells from apoptosis (Gennuso et al. 2004; Pallis et al. 2002), and transporter inhibition may thus promote cell death. Nevertheless, transient inhibition of efflux transporters by short-term administration of inhibitors may be a tolerable strategy to reverse or prevent drug resistance.

With regard to specific brain targeting, evidence exists that modulation of efflux transporter function may indeed enhance brain penetration of CNS therapeutics; however, transporter activity will also be affected in other blood–tissue barriers, haematopoetic cells, and excretory organs.

4.3 Prevention of Disease-Associated or Therapy-Induced Changes of the Blood–Brain Barrier

Expression of efflux transporters is regulated in a highly dynamic manner. This regulatory process can be considered as a mechanism that allows adaptation to changing requirements in detoxification and tissue protection. The regulation of expression has been most intensely studied for Pgp. Knowledge of the regulation of BBB efflux transporter activity is of particular interest because it may prepare the molecular basis for the development of strategies to specifically manipulate BBB function in order to improve pharmacotherapy of CNS diseases. This underlines the specific importance of further research focusing on the different mechanisms of regulation and their interaction.

A variety of xenobiotics including several APIs have been demonstrated to induce expression of multidrug transporters. In the treatment of brain cancer, induction of efflux transporter expression by chemotherapeutic drugs in tumor cells and BBB endothelial cells is a well recognized mechanism that limits drug concentrations at the target tumor cells and contributes to therapeutic failure (Lee and Bendayan 2004; Loscher and Potschka 2005a). The strong induction by anticancer drugs is probably due to their pronounced cytotoxic effects on cells and the induction of a cellular stress response. Orphan nuclear receptors have been recognized as master regulators of drug-induced changes in expression of metabolizing enzymes and of members of the multidrug transporter families (Masuyama et al. 2005). *The orphan nuclear receptor PXR/SXR* (termed pregnane X receptor [PXR] in rodents and steroid and xenobiotic receptor [SXR] in humans) proved to be expressed in rat brain capillaries (Bauer et al. 2004). Its functional relevance for regulation of efflux transporters in the BBB was indicated by the observation that the PXR ligand dexamethasone increased Pgp expression and Pgp-specific transport (Bauer et al. 2004). Thus, PXR/SXR may be a key xenobiotic sensor in brain capillary endothelial cells which mediates induction of Pgp. Targeting these xenosensors by administration of antagonists has been suggested as a means of overcoming therapy-induced resistance mechanisms (Ekins et al. 2007). The ongoing molecular characterization of the binding sites of the receptors has implications for future discovery of molecules that are more selective and potent than currently available antagonists.

Using intestinal and lung carcinoma cell lines it was demonstrated that induction of efflux drug transporters by xenobiotics and especially chemotherapeutics does not necessarily depend on PXR (Huang et al. 2006). Based on the fact that the group also demonstrated that the modes of regulation can be cell-specific, it is currently not clear if these data can be extrapolated to brain capillary endothelial cells. Baker et al. (2005) reported that *epigenetic changes in the MDR1 promoter* occur in response to chemotherapeutic drugs which then enhance the MDR phenotype. Dramatic changes in the temporal and spatial pattern of histone modifications occurred within the 5' hypomethylated region of MDR1, which directly correlated with MDR1 upregulation (Baker et al. 2005). Further research may create a basis for the identification of further targets for prevention of therapy-induced transporter overexpression.

Several CNS pathologies have been associated with changes in efflux transporter expression or function. Epilepsy, which is characterized by recurrent spontaneous seizures, is one of the most common neurological disorders. In animal models of epilepsy a transient increase in Pgp and MRP2 expression was observed in brain capillary endothelial cells, astroglia, and neurons after seizures, which indicates that seizures themselves can induce overexpression of drug transporters (Loscher and Potschka 2005a; Sisodiya 2003). This seizure-induced overexpression proved to be restricted to brain regions involved in seizure initiation and spread. These data are in line with investigations in human epileptogenic tissue dissected from pharmacoresistant patients during epilepsy surgery, which also

indicated high expression rates of efflux transporters (Loscher and Potschka 2005a; Sisodiya 2003). However, definite conclusions from these studies are hampered by the lack of adequate control tissue, because patients who are treated successfully do not generally undergo surgical resection of epileptogenic foci. The cellular mechanisms involved in seizure-induced overexpression of efflux transporters still need to be elucidated. With respect to the excessive glutamate release associated with seizures, it is of particular interest that glutamate proved to upregulate Pgp expression via an NMDA receptor mechanism (Zhu and Liu 2004). Recently, we were able to demonstrate that extracellular glutamate signals through the NMDA receptor and COX-2 in brain capillaries to increase BBB Pgp expression following seizures (Bauer et al. 2008). Consistent with our hypothesis, exposing isolated rodent brain capillaries to glutamate increased Pgp expression and transport activity. These increases were blocked by the NMDA receptor antagonist MK-801 and by the selective COX-2 inhibitor celecoxib. In rats, intracerebral microinjection of glutamate caused locally increased Pgp expression in brain capillaries. Moreover, using a pilocarpine status epilepticus rat model, we achieved an attenuation of seizure-induced increases in capillary Pgp expression by administration of the non-selective COX inhibitor indomethacin. These data suggest that it might be possible to enhance brain uptake of antiepileptic drugs and to overcome transporter-mediated resistance by COX inhibition (Bauer et al. 2008).

In accordance with the seizure-induced molecular changes at the BBB, an upregulation of Pgp has also been described following focal cerebral ischemia (Spudich et al. 2006). As enhanced glutamate release also is a hallmark during ischemic brain damage, this induction may also be related to glutamate release and subsequent activation of inflammatory events, and may be prevented using the same strategies as those already substantiated in an epilepsy model.

Further elucidation of the mechanisms involved in transporter regulation in CNS diseases may open avenues for new strategies to enhance brain penetration of CNS therapeutics. Apart from involved receptors or changes in the promotor region, a variety of mechanisms that contribute to cellular stress responses, including phospholipase C, proteinkinase C, mitogen-activated protein kinase cascades, mobilization of intracellular Ca^{2+}, cytokines, nuclear factor kappa B, and heat shock factor 1, regulate multidrug transporter genes such as *MDR1* (Ho and Piquette-Miller 2006; McRae et al. 2003; Shtil and Azare 2005; Tchenio et al. 2006). Using primary cultured rat brain endothelial cells to examine the effect of oxidative stress on expression of transporters, Felix and Barrand (2002) found a stress-induced increase in Pgp expression and function whereas no such alterations were observed for MRP1. Hartz et al. (2004, 2006) defined a signaling pathway as part of the innate immune response through which Pgp activity is rapidly modulated. Their findings suggested that the inflammatory cytokine tumor necrosis factor (TNF)-alpha reduces Pgp activity via TNF-R1 receptor activation, endothelin-1 release, and endothelin-B receptor signaling. All these findings complete our view of the regulatory mechanisms, and inspire research efforts for prevention of transporter-mediated resistance in CNS diseases.

5 Bypassing the Blood–Brain Barrier

5.1 Nano-Sized Carrier Systems and Drug Conjugates

An alternative approach which avoids compromising the protective function of efflux transporters is to bypass transporter molecules. Different strategies are followed in this regard including nanoparticle encapsulation (Huwyler et al. 1996; Kreuter 2001) or conjugation (Mazel et al. 2001) of transporter substrates.

Nano-sized carrier systems including polymers, emulsions, micelles, liposomes, and nanoparticles may deliver their content to the brain by passive targeting (de Boer and Gaillard 2007). The rate of distribution into the brain is often limited with these systems. The penetration rate will generally depend on the physicochemical features and the physiological conditions. A variety of compounds including several anticancer drugs have been formulated into nano-sized carrier systems. Efficacious delivery has been described in rodents. Whereas free doxorubicin did not induce any relevant effect in a brain tumor model in rats, liposomal doxorubicin increased the survival time (Sharma et al. 1997). Evidence exists that the first dose of doxorubicin thereby promotes subsequent brain uptake of further dosages due to a toxic effect on proliferating endothelial cells and reduction of the angiogenic factor VEGF (Zhou et al. 2002). Polymer-based particles were studied more rarely. They are in most instances formulated by adsorbing the drug onto the particle surface (Kreuter 1995). The particles are then phagocytosed into the cell where the drug is released. Enhanced brain uptake of doxorubicin has been demonstrated when the drug was loaded on the surface of solid poly(butyl cyanoacrylate) particles (Gulyaev et al. 1999). In a rat glioblastoma model long-term remission was achieved in 20% of the animals with the formulation (Steiniger et al. 2004).

In patients, therapeutic drug concentrations were reached in the central tumor mass following administration of liposomal daunorubicin (Zucchetti et al. 1999). In clinical trials the response rate to liposomal doxorubicin or daunorubicin was considered promising by the authors (Hau et al. 2004; Koukourakis et al. 2000a, b; Lippens 1999). However, conclusions are hampered by the fact that the patients received additional radiotherapy or other chemotherapeutic agents. Thus, further trials are necessary to clearly determine the therapeutic potential.

Active drug targeting strategies involve the application of a technology that utilizes endogenous transport mechanisms for site-specific delivery (de Boer and Gaillard 2007). Ligands for targeting may be conjugated to the drug itself or may be attached to the surface of drug-loaded particles. With respect to the BBB, ligand-mediated site-specific delivery involves receptor-mediated transcytosis systems at the BBB to reach extracellular or intracellular targets in the brain. Interestingly, targeting strategies can benefit from pathophysiological mechanisms, when the target is induced during the disease course. Gaillard et al. (2005) have identified a novel carrier protein for targeted delivery which makes use of the diphtheria toxin receptor, which is strongly induced under conditions of neuroinflammation such as

those occurring in many brain diseases, including Alzheimer's disease, Parkinson's disease, multiple sclerosis, ischemia, encephalitis, epilepsy, tumors, etc.

The carrier protein CRM197 is a nontoxic mutant of diphtheria toxin which specifically binds to the diphtheria toxin receptor resulting in receptor-mediated transcytosis and uptake into the brain (Gaillard et al. 2005). This delivery strategy can be applied using CRM197 drug conjugates or CRM197-coated drug-loaded liposomes.

The most widely characterized receptor-mediated transcytosis system for CNS targeting is the transferrin receptor (Pardridge 2002). Drug targeting to this receptor can be achieved either by using endogenous ligands or by using an antibody directed against the receptor (OX-26). The insulin receptor represents another receptor-mediated transcytosis system which has already been used for targeted delivery of drugs to the CNS (Pardridge 2005). An alternate approach is based on LRP1 and LRP2 receptors, which are known multiligand scavengers. Several ligands of these receptors including melanotransferrin, apolipoproteins, and aprotinin have already been used to promote brain targeting (de Boer and Gaillard 2007).

5.2 Intranasal Administration

Nasal drug administration is considered to provide one putative means for targeted CNS drug delivery (Graff and Pollack 2005). Three pathways are generally postulated for a drug administered to the nasal cavity to follow. These routes include direct delivery to the brain, e.g., along nerve sheaths, axonal transport along neurons, and entry into the blood from the nasal mucosa (Graff and Pollack 2005). To what extent a molecule passes along these routes and to what extent it is thus indeed targeted to the CNS critically depends on its chemical features and its formulation (Ugwoke et al. 2001). A recent meta-analysis of all published studies claiming evidence for direct nose-to-brain transport identified only two studies in rats which provide results that can be regarded as an indication for direct transport from the nasal mucosa to the CNS (Merkus and van den Berg 2007). The same analysis did not reveal any pharmacokinetic evidence supporting a claim that nasal administration of drugs in humans will result in an enhanced delivery to their target sites in the brain compared with intravenous administration of the same drug under similar dosing conditions. Thus, it is currently rather questionable whether intranasal administration can be considered as a means for efficacious brain targeting.

5.3 Intracerebral Administration

It is generally possible to achieve much higher concentrations of drug in the brain by direct administration into the cerebrospinal fluid or into the brain parenchyma

(Huynh et al. 2006). Intracerebroventricular or intrathecal drug infusion delivers drugs to the cerebrospinal fluid (CSF) thereby avoiding the BBB and hepatic metabolism. Following delivery to the CSF, APIs still have to pass the ependymal brain–CSF barrier. This is feasible for many small and lipophilic compounds. However, penetration into the parenchyma is limited due to tortuosity, transcapillary loss, cell uptake, and binding (de Boer and Gaillard 2007). Therefore, drugs administered to the CSF have minimal access to the parenchyma by diffusion. As a consequence, intraventricular administration of CNS drugs is considered particularly useful for meningioma treatment, as tumor metastasis is prevented by effects on cells floating in the CSF but is not applicable in glioma therapy (Huynh et al. 2006). The intrathecal administration route fails to result in drug accumulation in parenchymal structures deep within the brain, and is thus applicable rather for treatment of disseminated meningeal or spinal disease (Groothuis et al. 2000).

Implantable controlled release systems can be designed from both degradable and non-degradable polymers (Sawyer et al. 2006). Appropriately designed, polymers can provide reliable sustained release for periods of days to many years. As persistence of the delivery system will limit the clinical use, biodegradable polymers are more common than non-degradable systems. Controlled release systems are already used clinically for treatment of brain tumors (Sawyer et al. 2006). Intracranial implantation of a wafer loaded with carmustine (BCNU) following surgical debulking of the tumor was well tolerated in patients with malignant gliomas and resulted in a modest improvement of patient survival (Brem et al. 1995; Engelhard 2000). A general drawback of controlled release systems for different indications is that the local penetration of the drug is limited due to the restriction of diffusion by the brain parenchyma.

Convection-enhanced delivery was developed to deliver compounds throughout the brain to overcome the diffusion barrier seen with polymeric-controlled release (Bobo et al. 1994). The approach is based on continuous infusion which uses a convective flow to drive the API throughout a larger region of tissue (Huynh et al. 2006). In comparison with bolus injections to the brain parenchyma, the benefits are derived from the greater distribution and continued exposure due to the long infusion time (Sawyer et al. 2006). The technique has been used in chemotherapy, gene therapy, and immune therapy. Clinical use has been established in glioma patients with recurrence of tumors. Patients receive local infusions after surgical resection or infusion directly into the tumor. In clinical trials it has been demonstrated that convection-enhanced delivery is suitable for delivering agents to a large tumor volume. For example, clinical trials with delivery of an immunotoxin into glioblastoma tumors were able to achieve complete regression with minimal systemic toxicity in some patients (Husain et al. 2003).

Alternative approaches for direct delivery include gene therapy involving viral, lipid, polymer-based, and cell-based delivery strategies. For detailed information on these approaches readers are referred to reviews that focus on these techniques (de Boer and Gaillard 2007; Huynh et al. 2006).

6 Conclusions

In recent years awareness of the impact of brain efflux transporters on treatment of CNS diseases has progressively increased. Cumulative knowledge of the structure, function, localization, and substrate specificities of brain efflux transporters has helped to develop and validate strategies to deal with the activity of these transporters in a clinical setting. Several strategies for brain targeting of drugs are already applied clinically, especially for treatment of brain tumors. Recently, particular interest has arisen in the regulation of transporter expression or function in pathophysiological conditions, which may contribute to disease development or progression but may also influence the pharmacotherapeutic outcome. Further research may provide new approaches which prevent a strengthening of BBB function in CNS diseases, and may thereby prevent development of pharmacoresistance.

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