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Drug transport to the brain: Key roles for the efflux pump P-glycoprotein in the blood–brain barrier

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Abstract

1. The blood–brain barrier (BBB) contributes to brain homeostastis and fulfills a protective function by controlling the access of solutes and toxic substances to the central nervous system (CNS). The efflux transporter P-glycoprotein (P-gp) is a key element of the molecular machinery that confers special permeability properties to the BBB. 2. P-gp, which was initially recognized for its ability to expel anticancer drugs from multidrug-resistant cancer cells, is strongly expressed in brain capillaries. Its expression in the BBB limits the accumulation of many hydrophobic molecules and potentially toxic substances in the brain. 3. The purpose of this review is to summarize the current state of knowledge about the expression of P-gp, its cellular localization as well as its possible functions in the BBB.

Keywords: Blood–brain barrier; P-Glycoprotein; Multidrug resistance; Endothelial cells; Caveolae

1. Introduction: The blood–brain barrier (BBB) and P-glycoprotein (P-gp)

The brain is shielded against potentially toxic substances by the presence of two barrier systems: the BBB and the blood–cerebrospinal fluid barrier (BCSFB). Because its surface area is approximately 5000-fold greater than that of the BCSFB, the BBB is considered to be the major route for the uptake of endogenous and exogenous ligands into the brain parenchyma (Pardridge, 1999; Kusuhara and Sugiyama, 2001). The BBB is formed by brain capillary endothelial cells (BCECs), which are closely sealed by tight junctions. In addition, brain capillaries possess few fenestrae and few endocytic vesicles as compared to capillaries of other organs (Pardridge, 1999). BCECs are surrounded by extracellular matrix, astrocytes, pericytes and microglial cells. The close association of endothelial cells with the astrocyte foot processes and the basement membrane of capillaries is important for the development and maintenance of the BBB properties that permit tight control of blood–brain exchange (Pardridge, 1999; Tsuji and Tamai, 1998; Kusuhara and Sugiyama, 2001).

The tight junctions in the BBB prevent significant passive movement of small hydrophilic molecules from the blood to the brain, but specialized transport systems mediate the entry of essential substances such as glucose, amino acids, choline, monocarboxylic acids, amines, thyroid hormones, purine bases and nucleosides (Tsuji and Tamai, 1999; Kusuhara and Sugiyama, 2001). Larger hydrophilic molecules do not cross the BBB to any significant extent aside from specific proteins such as transferrin, lactoferrin and low-density lipoprotein, which are taken up by receptor-mediated endocytosis. Elegant studies have permitted significant progress in the characterization of the transcytosis of these proteins (Dehouck et al., 1997; Fillebeen et al., 1999). The BBB is frequently a rate-limiting factor for the penetration of drugs into the brain. The factors determining passive drug entry into the central nervous system (CNS) have been recently reviewed (Habgood

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et al., 2000). Due to the lipidic nature of cell membranes, lipid solubility is an important determinant of passive BBB permeability. The ability to partition into an organic solvent relative to a physiological buffer (octanol/buffer partition coefficient) is a common measure of lipophilicity. The overall hydrophilic/lipophilic balance of a molecule appears to be a better predictor of BBB permeability than the octanol/buffer partition coefficient. Molecular size, to which the rate of diffusion of a solute is inversely related, appears to be relevant for hydrophilic compounds, but does not significantly influence the BBB permeability of lipophilic compounds. Binding to plasma proteins, ionization at physiological pH (pK_a), affinity and capacity for transport systems and potential BBB/cerebral metabolism are also important. As discussed below, the presence of the efflux transporter P-gp in the BBB prevents significant accumulation of many hydrophobic molecules or drugs in the CNS (van Asperen et al., 1997; Schinkel, 1999).

2. The efflux transporter P-gp

P-gp is a membrane transporter of the ABC (ATP binding cassette) superfamily that was initially described in the field of cancer research (Biedler and Riehm, 1970; Ling and Thompson, 1974). Its expression was associated with inherent or acquired multidrug resistance (MDR) phenotype by cancer cells (Biedler and Riehm, 1970; Dano, 1973). This phenotype renders the cells resistant not only to the agent to which they are exposed but also to other agents of unrelated structure or function. By actively preventing uptake and increasing cellular efflux in an ATP-dependent manner, P-gp prevents the intracellular accumulation of anticancer agents and thereby reduces their cytotoxicity (Endicott and Ling, 1989; Gottesman and Pastan, 1993).

In humans, P-gp is encoded by two *MDR* [*MDR1* and *MDR3* (also called *MDR2*)] genes (Callen et al., 1987; Chin et al., 1989). Three genes have been identified in rodents [*mdr1a*, *mdr1b* and *mdr2*] (Gros et al., 1986; Ueda et al., 1986). MDR1 in humans and both mdr1a and mdr1b in rodents confer the resistance phenotype, whereas human MDR3 and rodent mdr2 do not. Instead, MDR3 and mdr2 appear to be involved in the transport of phosphatidylcholine across the canalicular membrane of hepatocytes (Smit et al., 1993; Ruetz and Gros, 1994). However, it was recently shown that human MDR3 binds and transports a subset of MDR1 P-gp substrates, albeit inefficiently (Smith et al., 2000). This may explain why MDR3 is not detectably involved in MDR.

3. P-gp substrates and reversal agents

The first molecules identified as P-gp substrates were generally from natural sources, either plants or microorganisms. Their structures often included hydrophobic regions, planar aromatic domains in particular, as well as tertiary amino groups or positive charges at physiological pH (Seelig and Landwojtowicz, 2000). Among the transported anticancer agents are vinca alkaloids, epipodophyllotoxins, anthracyclines, taxanes and antibiotics such as erythromycin and tetracycline (Gottesman and Pastan, 1993; Ambudkar et al., 1999; Silverman, 1999). Increasingly, molecules other than anticancer agents have been identified as P-gp substrates. For example, P-gp transports digoxin, dexamethasone, anti-HIV protease inhibitors, opioids, fluorescent dyes (rhodamine 123), calcein-AM and cyclic and linear peptides such as gramicidin D and valinomycin (Ambudkar et al., 1999; Silverman, 1999; Thompson et al., 2000). Endogenous substrates such as steroids (cortisol and aldosterone), cytokines (IL-2, IL-4 and IFN-γ) and bilirubin were also shown to be transported by P-gp (van Kalken et al., 1993; Watchko et al., 1998; Drach et al., 1996). Furthermore, natural products such as curcumin (Romiti et al., 1998) and epigallocatechin gallate, the major polyphenol present in green tea, interact with the substrate binding site of P-gp (Jodoin et al., 2002).

Reversal agents are molecules that restore sensitivity to anticancer agents in drug-resistant cancer cells by inhibiting the transport activity of P-gp. Among first-generation agents are calcium channel blockers, calmodulin antagonists, quinolins, steroids, immunosuppressive agents, antibiotics and detergents (Ford and Hait, 1990; Lum et al., 1993; Raderer and Scheithauer, 1993). However, most of these agents produce significant toxicities when used at concentrations sufficient to inhibit P-gp. This has led to the development of second- and third-generation P-gp modulators such as the non-immunosuppressive analogue SDZ PSC 833 (valspodar) (Twentyman, 1992), biricodar VX-710 (Germann et al., 1997), the acridone carboxamide derivative GF120918 (GG918) (Hyafil et al., 1993), the substituted dibenzosuberane molecule LY335979 (Dantzig et al., 1996), the novel substituted diarylimidazole OC144-093 (Newman et al., 2000), the diketopiperazine derivative XR9051 and the anthranilic acid derivative XR9576 (Mistry et al., 2001). A number of these second- and third-generation agents are now in clinical trials, and initial studies have reported some clinical benefit from the use of P-gp modulators such as SDZ PSC 833 (Dorr et al., 2001). The clinical efficacy of these reversal agents remains to be established, not only with regard to overcoming tumor resistance towards chemotherapy, but also for other factors such as bypassing P-gp in the BBB.

4. Localization of P-gp

The expression of P-gp in human tissues has been the object of numerous investigations utilizing various experimental approaches such as in situ hybridisation and immunocytochemistry (Fojo et al., 1987; Thiebaut et al., 1987; Cordon-Cardo et al., 1990). MDR1 P-gp is present in many

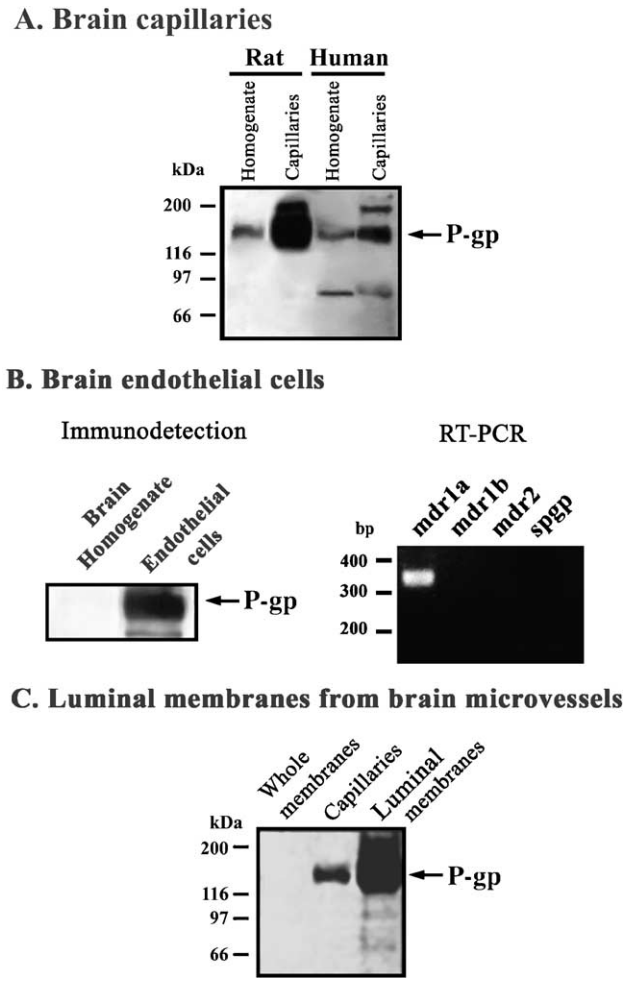

Fig. 1. Immunodetection of P-gp. (A) P-gp was immunodetected by Western blot analysis using the monoclonal antibody C219 in brain homogenate and capillaries from rat and human. (B) Endothelial cells were isolated with magnetic microbeads cross-linked to an anti-PECAM-1 antibody. P-gp was immunodetected in the rat brain homogenate and in the enriched endothelial cell fraction. RT-PCR was also performed with RNA isolated from the selected endothelial cells with primers directed against *mdr1a*, *mdr1b*, *mdr2* and spgp. (C) P-gp was also detected in whole brain membranes, brain capillaries and isolated luminal membranes of the brain vascular bed.

human tissues such as liver, kidney, intestines and adrenal glands as well as in blood–tissue barriers including the placenta, testis capillaries and brain capillaries (Cordon-Cardo et al., 1989; Jetté et al., 1993). We have used isolated brain capillaries to further characterize the expression of P-gp in various species including human, mouse and rat (Jetté et al., 1993; Jetté et al., 1995b). As shown in Fig. 1A, P-gp is highly expressed in capillaries isolated from rat and human brain. The isoform of P-gp expressed in mouse brain capillaries was determined by Western immunoblotting using isoform-specific antibodies. We found that P-gp mdr1a is expressed in brain capillaries isolated from mouse, whereas mdr1b and mdr2 isoforms were not detected (Jetté et al., 1995b). Analysis of *mdr* RNA by Northern blots showed that both *mdr1a* and *mdr1b* are present in mouse brain (Croop et al., 1989). However, *mdr1a* was specifically detected by RT-PCR in rat brain capillaries, whereas *mdr1b* was present only in rat brain parenchyma (Regina et al., 1998). In human brain, MDR1 is the prominent P-gp isoform (Cordon-Cardo et al., 1989, Thiebaut et al., 1987; Schinkel, 1999).

Using immunoelectron and confocal microscopy, it has been reported that P-gp is localized in endothelial cells lining the BBB (Tsuji et al., 1992; Stewart et al., 1996). To further characterize P-gp in the brain vasculature, we developed a technique for isolating endothelial cells with magnetic microbeads cross-linked to an antibody directed against platelet–endothelial cell adhesion molecule-1 (Demeule et al., 2001a). P-gp was strongly enriched (59-fold) in the positive endothelial cell fraction from brain

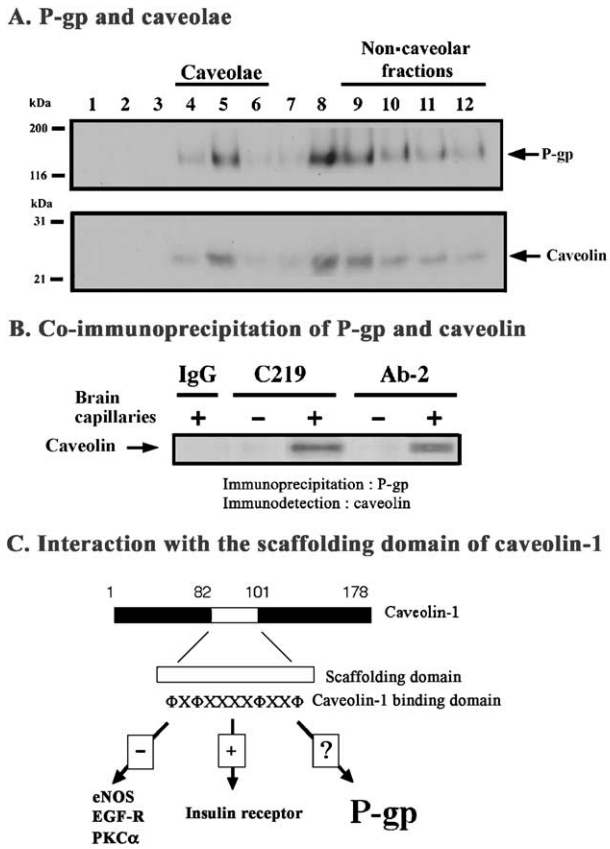

Fig. 2. Distribution of caveolin and P-gp in caveolae. (A) Brain capillaries from rat brain were subjected to subcellular fractionation by a standard procedure using a sucrose gradient to isolate caveolae. Gradients were fractionated by collecting 1-ml fractions. P-gp and caveolin were immunodetected in these fractions. Fractions 4–6 correspond to caveolar fractions, whereas fractions 9–12 are non-caveolar fractions. (B) P-gp and caveolin were co-immunoprecipitated from brain capillaries. P-gp was first immunoprecipitated using two antibodies directed against P-gp (C219 and anti-MDR1 Ab-2). A control immunoprecipitation was also performed with a nonspecific IgG. Following the P-gp precipitation, caveolin was detected in these samples. (C) Representation of the consensus scaffolding-binding domain of caveolin-1. The interactions of caveolin-1 with eNOS, EGF-receptor and PKCα cause down-regulation of these proteins, whereas the interaction with caveolin-1 increases the activity of the insulin receptor. In the case of P-gp, the effect of its interaction with caveolin-1 is unknown.

(Fig. 1B) and was absent in the negative fraction in which the glial fibrillary acidic protein (GFAP), an astrocyte marker, was present. It was shown by RT-PCR analysis that the *mdr1a* gene is preferentially expressed in this enriched EC fraction from the brain (Fig. 1B). At the subcellular level, P-gp was localized in isolated luminal membranes from the brain vascular endothelium in rat (Fig. 1C) (Beaulieu et al., 1997). Luminal membranes from brain microvessels were isolated by modifying their density via perfusion with a cationic colloidal silica. Subsequent coating of the silica with polyanionic cross linker allowed the isolation of luminal membranes by centrifugation. Using this procedure, P-gp in luminal membranes were enriched 17-fold as compared to brain capillaries and 400–500 fold relative to membranes from whole brain. In these isolated luminal membranes, GFAP showed a very weak enrichment (1.4-fold over brain capillaries) indicating minimal contamination by astrocytes (Beaulieu et al., 1997). In contrast, it was shown by confocal immunofluorescent microscopy that P-gp and GFAP are co-localized in isolated human brain capillaries (Golden and Pardridge, 1999). From these results, it was suggested that P-gp is localized at astrocyte foot processes on the antiluminal side of brain microvasculature instead of at the luminal side. This model is in contradiction with previous studies where MDR1 was detected in endothelial cells both of normal cerebral capillaries and of newly formed microvessels of gliomas (Sugawara et al., 1990; Toth et al., 1996; Sawada et al., 1999). Taken together, most of the published data supports the contention that P-gp is principally expressed at the luminal membranes of BCECs in mammals.

P-gp has also been localized in a specialized microdomain of plasma membranes called caveolae, where it appears to play an important role in the development of resistance in MDR cells (Lavie et al., 1998; Yang et al., 1998; Demeule et al., 2000). Caveolae are flask-shaped plasma membrane invaginations involved in many cellular events such as signal transduction, lipid and protein sorting, endocytosis and potocytosis (Shaul and Anderson, 1998). In capillary endothelial cells, caveolae are involved in the transport of macromolecules across the cells by transcytosis (Schnitzer et al., 1994). Caveolins are the structural proteins of caveolae for which three genes have been identified (caveolin-1, -2 and -3). Caveolin-1 and -2 are primarily expressed in adipocytes, endothelial cells and fibroblastic cell types, whereas caveolin-3 is expressed in myocytes (Okamoto et al., 1998). In the brain, caveolin-1 and -2 are principally expressed in capillary endothelial cells, and caveolin-3 is predominantly expressed in astroglial cells (Ikezu et al., 1998). We have isolated caveolae from brain capillaries by flotation of low-density microdomains on sucrose gradients where caveolin-1 was enriched. Interestingly, we found that P-gp is co-localized with caveolin-1 in these microdomains (Fig. 2A) (Demeule et al., 2000). In addition to co-localization of P-gp with caveolin-1 on gradients, co-immunoprecipitation studies indicated that a portion of P-gp molecules physically interacts with caveolin-1 (Fig. 2B).

P-gp contains a consensus caveolin-binding motif that binds to the scaffolding domain of caveolin (Fig. 2C). Three related caveolin-binding motifs are known (i.e., ΦXΦXXXXΦ, ΦXXXXΦXXΦ and ΦXΦXXXXΦXXΦ where Φ is a phenylalanine, tyrosine or tryptophan residue and X is any aminoacyl residue) (Couet et al., 1997). The scaffolding domain of caveolin-1 is involved in interactions with numerous proteins and was shown to negatively regulate some signaling molecules localized in caveolae, including eNOS, protein kinase C and epidermal growth factor receptor (Fig. 3B) (Okamoto et al., 1998). In contrast to this, interaction of caveolin-1 with the insulin receptor increases insulin-stimulated phosphorylation of downstream targets (Yamamoto et al., 1998). Although it has been

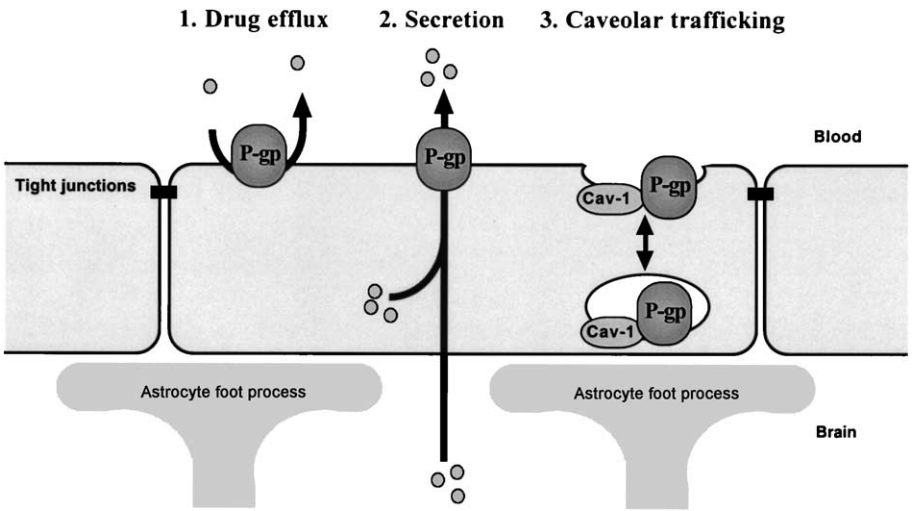

Fig. 3. Physiological roles of P-gp in brain capillaries. Schematic diagram of P-gp in the BBB: (1) P-gp limits the penetration of xenobiotics into the brain, (2) P-gp contributes to brain or endothelium secretion or excretion, and (3) P-gp might be involved in caveolar trafficking.

shown that the disruption of caveolae alters drug transport by P-gp in a cell-type specific manner (Luker et al., 2000), the functional consequence of caveolin-1 and P-gp association remains to be established.

5. P-gp in brain tumors

Clinical progress in the treatment of brain tumors has been slow and one of the problems impeding treatment of these tumors is their weak response to anticancer drugs. The effectiveness of chemotherapy and immunotherapy in the CNS is often impaired by the BBB, which physically restricts the entry of large and/or hydrophilic drugs into the brain. The low response to chemotherapy may also depend on tumor blood flow, the integrity of the blood–tumor barrier and inherent or acquired MDR phenotype in cancer cells (Béliveau et al., 1998). As P-gp plays a major role in the defense of the organism against xenobiotics at the BBB (Schinkel, 1999), the determination of P-gp levels in brain tumors and peritumoral tissue is crucial for evaluating the long-term efficacy of chemotherapy. We investigated the presence of P-gp in various human brain tumors, including low- and high-grade gliomas, brain metastases and benign brain tumors (Demeule et al., 2001b). P-gp was detected in brain tumors by Western blots using the monoclonal antibody anti-MDR1 Ab-2, which specifically recognizes MDR1 and not MDR3. This approach allows quantitative description of protein expression in a tissue and eliminates cross-reactivity with other proteins, therefore, reducing the number of false positive results.

5.1. Malignant brain tumors

The expression levels of MDR1 P-gp in most samples from various malignant brain tumors (low- and high-grade gliomas) were similar to normal brain levels. These results are in agreement with previous studies, which reported the presence of P-gp in resistant and partially chemosensitive glioblastomas by immunohistochemistry using mAb C219 (Becker et al., 1991; Henson et al., 1992; Leweke et al., 1998). These results suggest that the poor response of brain tumors to many anticancer drugs may be related to the presence of this protein in cell populations of the primary brain tumors and that P-gp may be seen as a negative factor for predicting the outcome of patients with brain tumors. In addition, these findings also suggest that P-gp expression is maintained in both low- and high-grade gliomas. The widespread expression of P-gp in these tumors may reflect an intrinsic resistance to anticancer drugs.

5.2. Benign brain tumors

Among the brain tumors that we investigated, the highest levels of P-gp were found in meningiomas, where they were at least 24-fold higher than in normal brain. Since none of the patients with meningiomas had received chemotherapy or radiotherapy prior to surgery, the high P-gp expression in these tumors could not have been induced by any treatment. The role of chemotherapy in the treatment of recurrent benign meningiomas or malignant meningiomas is not well defined (Greenberg et al., 1999a). However, because of the slow growth of these tumors, agents that affect cell replication would not be likely to have a significant impact on these tumors. The high P-gp expression observed in benign meningiomas suggests that these tumors may also have a poor response to chemotherapeutic agents transported by P-gp.

5.3. Brain metastases

Brain metastases occur in 20–40% of cancer patients and the highest prevalence at autopsy are from melanomas (40–68%) and lung cancers (21–36%) (Greenberg et al., 1999b). Strikingly, brain metastastes from melanomas and lung adenocarcinomas exhibit only 5% and 40%, respectively, of the P-gp levels found in normal brain. Metastatic malignant melanomas are recognized for their poor response to chemotherapy, whereas some effects of chemotherapy have been observed for lung adenocarcinomas (Savas et al., 1999; Greenberg et al., 1999a). The low expression of P-gp in these brain metastases suggests that MDR mechanisms other than P-gp could be responsible for their poor response to chemotherapy. The lack of P-gp expression in primary lung tumors and corresponding brain metastases also indicates that these brain metastases did not acquire the levels of P-gp expression found in normal brain tissue.

Previous immunohistochemical analyses showed that most gliomas and, more specifically, endothelial cells within the gliomas, stained positively for MDR1 P-gp (Toth et al., 1996; Sawada et al., 1999). These studies support the concept that clinical drug resistance may be caused by P-gp expression not only in cancer cells but also in the capillary endothelial cells of brain tumors. The role of the BBB in the low efficacy of chemotherapy is still unclear. Alterations in the brain capillary ultrastructure have been described leading to an increase in the microvascular permeability in gliomas. In contrast, it has been reported that the neovasculature of even high-grade tumors preserves partial BBB permeability properties at the cellular level (Sawada et al., 2000), and that the BBB at the tumor periphery is still intact. In addition, P-gp, one of the best phenotypic marker of the BBB, is expressed at the same levels in all primary tumors as in normal brain indicating that brain tumors retain an important characteristic of the BBB, which allows to restrict the brain uptake of chemotherapeutic agents. Thus, BBB, especially at the edge of tumors, remains a formidable obstacle for drug distribution to brain regions that have been infiltrated by neoplastic cells (Bertossi et al., 1997).

6. Role of P-gp in brain capillaries

In brain capillaries, P-gp appears to play an important role in preventing many hydrophobic molecules from crossing the BBB and reaching the CNS. However, the exact physiological function of P-gp in the BBB is not completely understood. A growing body of evidence links P-gp to physiological roles distinct from its initially recognized function as a drug efflux system (Fig. 3).

6.1. Drug efflux

Numerous reports have provided functional evidence for P-gp-mediated drug efflux at the BBB. The interaction of drugs with P-gp in rat brain capillaries has been demonstrated by photoaffinity labeling (Jetté et al., 1995a). The generation of transgenic mice with a disruption of the *mdr1a* gene has provided a pharmacological tool in the study of P-gp function in the BBB (Schinkel et al., 1994; Schinkel, 1999). These mice are viable, fertile and do not display obvious phenotypic abnormalities, indicating that this protein is not essential to their vital functions. However, P-gp substrates accumulate in the brains of these mice to a much greater extent than in wild-type animals and they are more sensitive to central neurotoxicity. For example, the knockout mice are 50–100 times more sensitive to the neurotoxic effects of the pesticide ivermectin. The accumulation of this drug in brain tissue of *mdr1a(−/−)* mice was increased 80–100-fold as compared to control mice. Recent application of in situ brain perfusion to wild-type and P-gp-deficient [*mdr1a(−/−)*]-mice made it possible to assess the influence of P-gp on brain uptake of substrates without potentially confounding differences in systemic pharmacokinetics upon P-gp distribution (Cisternino et al., 2001). In summary, P-gp appears to be a major transporter at the BBB that acts as a guardian of the CNS by preventing the accumulation of many drugs in the brain.

6.2. Secretion or excretion

In addition to its guardian role, P-gp is involved in the excretion of toxic compounds by renal proximal tubules and hepatic canalicular membranes (Cordon-Cardo et al., 1990; Thiebaut et al., 1987), and in the secretion of endogenous molecules from adrenal glands (van Kalken et al., 1993). Thus, P-gp could fulfill a similar function in the BBB and be responsible for the secretion and/or excretion of brain-derived substances or metabolites into the blood (brain secretion). It could also be involved in the secretion of molecules from the endothelium itself (capillary secretion). The results of a recent study suggest that P-gp may be involved in the release of neuroactive substances from the brain directly into the systemic blood following an intracerebroventricular injection (King et al., 2001). Finally, it has been demonstrated that β-amyloid is transported across the plasma membrane of P-gp-enriched vesicles in an ATP- and P-gp-dependent manner, suggesting that β-amyloid might be an endogeneous substrate for P-gp in brain (Lam et al., 2001).

6.3. Novel functions for P-gp

P-gp has been found to be involved in cytokine secretion from lymphocytes (Drach et al., 1996), chloride channel activity (Idriss et al., 2000), dendritic cell migration (Randolph et al., 1998) and steroid secretion from adrenal glands (van Kalken et al., 1993). Recent studies have opened new research areas investigating the role of P-gp in the regulation of cell death (Johnstone et al., 2000), as well as its involvement in cell differentiation as demonstrated in murine bone marrow stem cells transfected with the *MDR1* gene (Bunting et al., 2000).

As mentioned above, the function of P-gp in caveolae of the endothelial cells of the BBB and the effect of caveolin-1 on its activity are still unknown. However, the findings of different groups suggest involvement of P-gp in lipid transport. Specifically, MDR2 P-gp acts as a phosphatidylcholine flippase and MDR1 P-gp has been reported to transport sphingomyelin (SM), phosphatidylserine and phosphatidylethanolamine (Borst et al., 2000a). Furthermore, cholesterol interacts directly with the substrate binding site of P-gp, suggesting that cholesterol may be transported by MDR1 P-gp (Wang et al., 2000). Moreover, caveolin-1 binds cholesterol and mediates its efflux within caveolae and via a recently identified cytosolic caveolin-1 complex comprising heat-shock protein 56, cyclophilin A and cyclophilin 40, which carries cholesterol to the plasma membrane caveolae (Uittenbogaard et al., 1998). Since P-gp is present in caveolae, which are SM and cholesterol-rich membrane domains, it could mediate the translocation of cholesterol from the inner leaflet to the outer leaflet of membrane, as proposed for SM (Johnstone et al., 2000). This hypothesis must be verified to have a better understanding of the role of P-gp in caveolae at the BBB. These findings suggest that P-gp could be a key player in the trafficking of lipids in these important membrane microdomains involved in several crucial pathways in cell proliferation, signal transduction and transcytosis.

7. Other MDR transporters in brain capillaries

It has been reported that efflux transporters other than P-gp are also expressed in brain capillaries. For instance, members of the multidrug resistance-associated protein (MRP) family have been detected at the BBB site. In humans, nine MRP homologues have been identified (Borst et al., 2000b; Bera et al., 2001; Bera et al., 2002). All the members of the MRP family are distributed throughout most human tissues (Flens et al., 1996). MRP1, which was first described by Cole et al. (1992), was immunodetected by Western blots in human and rat

Table 1
Identification of caveolin-binding motifs in the multidrug resistance proteins P-gp, Spgp and MRPs

MDR transporters	Number of caveolin-binding motifs	Sequence localization[a]
P-gp (human)		
MDR1	1	37
MDR3	2	767, 919
Spgp (rat)	2	328, 335
MRPs (human)		
MRP1	6	31, 72, 156, 350, 535, 1110
MRP2	3	39, 164, 1037
MRP3	2	237, 560
MRP4	1	703
MRP5	0	
MRP6	2	62, 137

[a] First amino acid of the caveolin-binding sequence motif.

choroid plexus but the presence of MRP1 in endothelial cells of brain capillaries remains controversial. In animal models, Western blot and RT-PCR analysis suggest that MRP1 is expressed in isolated rat brain capillaries, primary cultured rat, porcine and BCECs and immortalized rodent BCECs (Huai-Yun et al., 1998; Regina et al., 1998; Kusuhara et al., 1998). However, in isolated human brain capillaries, no expression of MRP1 was observed by immunohistochemistry (Seetharaman et al., 1998). The canalicular multispecific organic anion transporter (cMOAT or MRP2) was not detected in rat brain capillaries by Western blot (Demeule et al., 1999). Recently, MRP1, 4, 5 and 6 were shown to be expressed in primary BCECs by RT-PCR analysis and in a capillary enriched brain extract (Zhang et al., 2000). Caveolin-binding motifs are also present in ABC transporters other than P-gp, such as the MRPs and the sister of P-glycoprotein (spgp) (Table 1). In spite of the fact that these transporters, aside from MRP5, contain at least one caveolin-binding motif, their localization in caveolae or their association with caveolin remains to be investigated in order to determine whether these are common features for MDR transporters or if they are restricted to P-gp. In addition, MRP mRNA levels appeared to be closely associated with resistance to etoposide, adriamycin and vincristine in human glioma cell lines derived from patients (Abe et al., 1994). Recently, levels of MDR1 and MRP1-4 genes were compared between normal brain tissue and malignant gliomas (Haga et al., 2001). The expression of both MDR1 and MRP2 were similar in normal brain and tumors, whereas MRP1 and MRP3 expression increased with tumor grade. Therefore, some of the MRPs may also confer intrinsic MDR activity in human gliomas.

In conclusion, P-gp in the capillary endothelial cells of the BBB restricts the CNS accumulation of many drugs including chemotherapeutic agents. Different approaches are currently being investigated to bypass this barrier such as P-gp inhibitors and reversible BBB opening (Abbott and Romero, 1996; Jolliet-Riant and Tillement, 1999). In addition, single-nucleotide polymorphism in the P-gp MDR1 gene has been reported to affect the pharmacokinetics of digoxin, most probably through both an increase in intestinal absorption and a decrease in renal excretion (Hoffmeyer et al., 2000; Cascorbi et al., 2001). These recent findings suggest that MDR1 polymorphisms could potentially modify drug distribution to the CNS. Finally, the physiological functions of MDR1 P-gp and other efflux pumps at the BBB remain to be established, which would better enable us to understand the molecular phenomena underlying drug delivery to the normal brain and to brain tumors.

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