The Blood-Brain Barrier and Cancer: Transporters, Treatment, and Trojan Horses

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Abstract Despite scientific advances in understanding the causes and treatment of human malignancy, a persistent challenge facing basic and clinical investigators is how to adequately treat primary and metastatic brain tumors. The blood-brain barrier is a physiologic obstruction to the delivery of systemic chemotherapy to the brain parenchyma and central nervous system (CNS). A number of physiologic properties make the endothelium in the CNS distinct from the vasculature found in the periphery. Recent evidence has shown that a critical aspect of this barrier is composed of xenobiotic transporters which extrude substrates from the brain into the cerebrospinal fluid and systemic circulation. These transporters also extrude drugs and toxins if they gain entry into the cytoplasm of brain endothelial cells before they enter the brain. This review highlights the properties of the blood-brain barrier, including the location, function, and relative importance of the drug transporters that maintain this barrier. Primary and metastatic brain malignancy can compromise this barrier, allowing some access of chemotherapy treatment to reach the tumor. The responsiveness of brain tumors to systemic treatment found in past clinical research is discussed, as are possible explanations as to why CNS tumors are nonetheless able to evade therapy. Finally, strategies to overcome this barrier and better deliver chemotherapy into CNS tumors are presented.

Despite the dramatic advances in understanding the molecular basis for carcinogenesis and the development of new targeting agents to treat malignancies, a critical challenge that continues to face cancer researchers is overcoming the sanctuary for primary and metastatic disease found within the central nervous system (CNS). Brain metastases occur in a significant percentage of patients with common malignancies, with 5-year cumulative incidence rates of 16% in lung cancer patients, 7% of breast cancer patients, and 5% of patients with colon cancer (1). In diseases such as melanoma, the incidence of brain metastatic disease is reported to be as high as 55% (2). Autopsy studies show that in patients who die from cancer, up to 25% of them develop brain metastases (3).

The incidence of brain metastatic disease is on the rise (4). This could be due to a number of factors, including earlier brain screening for CNS disease in cancers known to spread to the brain; improved and more widely available radiological techniques such as magnetic resonance imaging; and improved therapies to treat systemic disease, which are prolonging survival and, in turn, increasing the risk of developing metastases to the brain. The irony is that as our therapies are improving clinical outcomes and prolonging survival, the incidence of CNS disease is on the rise. Furthermore, primary brain malignancies are intrinsically resistant to most chemotherapies for reasons that are poorly understood. These realities demand that we better understand, and learn how to treat, CNS malignancy.

This CNS sanctuary for metastatic as well as primary brain disease is formed by the blood-brain barrier (BBB), a mechanism found across species that protects the brain from exposure to toxins, both endogenous and exogenous. This barrier prevents many of our traditional and newer drugs from crossing from the circulation into the brain parenchyma. Significant research has gone into understanding the mechanisms that form this barrier, as well as investigating means of circumventing the barrier to deliver therapy. In this review, we will present research findings on the physiologic properties of the BBB, the critical role of drug transporters in forming the BBB, as well as disruptions in the BBB found at metastatic tumor sites. Finally, current and future strategies to increase chemotherapy delivery to the brain will be presented.

Physiologic Properties of the BBB

A number of morphologic, physiologic, and functional characteristics of the BBB ensure that endogenous and exogenous substrates in the general circulation do not readily cross into the brain parenchyma. These characteristics of brain endothelium differentiate these cells from those found in endothelial beds in other organs in the periphery (Fig. 1). To better understand the unique properties of the BBB, it is useful to review the characteristics of capillary beds in general. In endothelial cells forming capillary beds in the periphery, pores are formed between cells through intercellular clefts (Fig. 1A).

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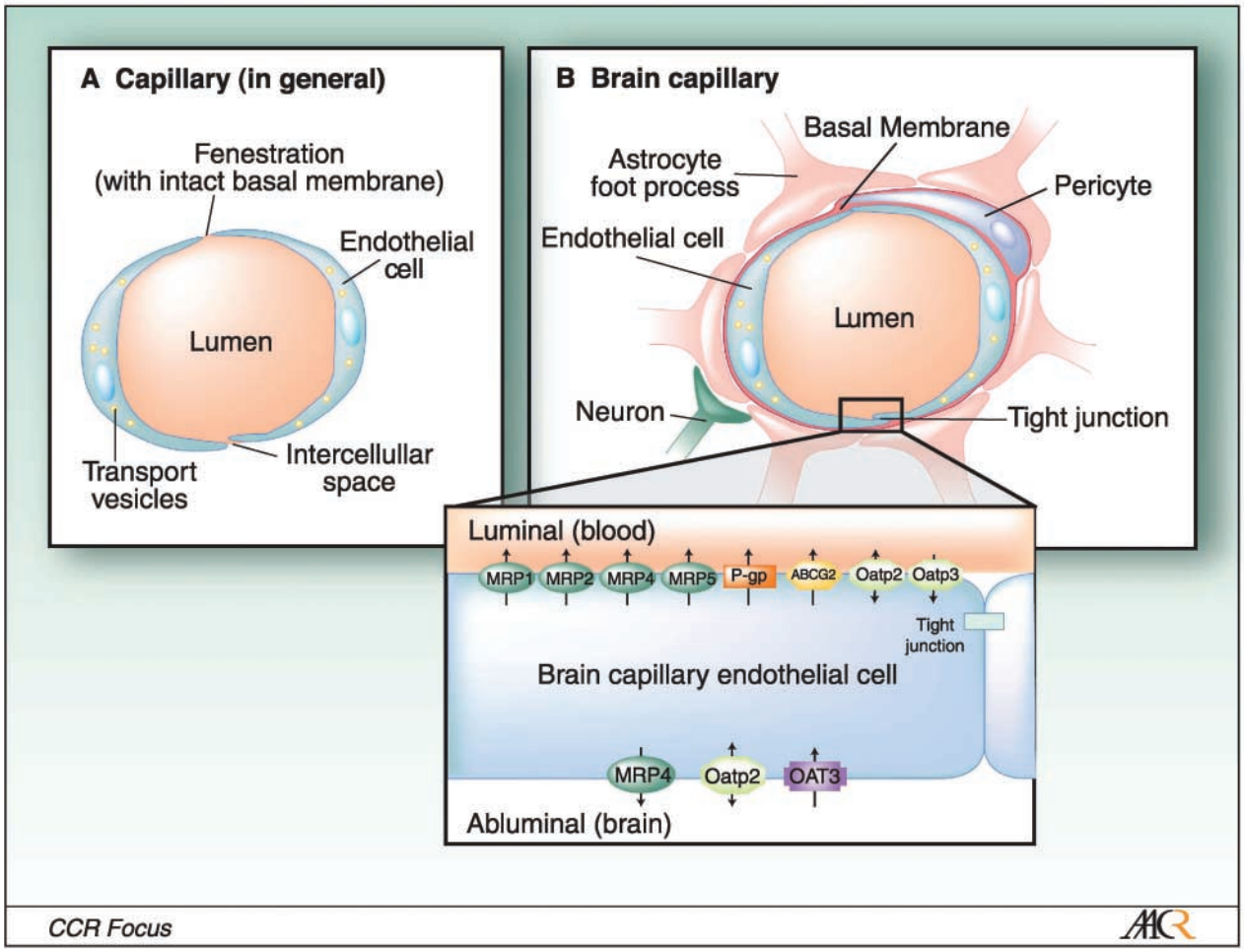

Fig. 1. Schematic comparison of a brain capillary (*B*) with a capillary in the periphery (*A*). Because the BBB has one major objective—to protect neurons from systemically circulating potentially cytotoxic agents—brain capillaries form a very tight barrier clearly distinct from capillaries in other organs. Brain capillaries lack fenestration and have low pinocytosis and only a few pinocytotic vesicles, but greater numbers and greater volumes of mitochondria than seen in a variety of peripheral tissue capillaries. The BBB is formed by capillary endothelial cells, surrounded by a basal membrane and astrocytic perivascular end-feet. Tight junctions present between the cerebral endothelial cells form a diffusion barrier, which severely restrict penetration of water-soluble compounds, including polar drugs, into the brain. Astrocytic end-feet tightly ensheath the vessel wall and seem to be critical for the induction and maintenance of the endothelial barrier. Furthermore, pericytes intimately embrace the brain capillaries and seem to contribute to the development, maintenance, and regulation of the BBB. Nerve fibers from peripheral nerve ganglia and intrinsic brain neurons regulate cerebrovascular tone resulting in functional "neurovascular units," which has an important role in maintaining a precisely regulated microenvironment for reliable neuronal activity. Due to the tightness of the endothelial barrier, paracellular transport of substances is negligible under physiologic conditions. Consequently, drugs and other substances can enter the brain only by passive transcellular diffusion, which is restricted to lipid-soluble agents, by receptor-mediated transcytosis, such as described for insulin and transferrin, or by specific carrier systems such as described for glucose, amino acids, purine bases, nucleosides, choline lactate and other substances. The enhanced illustration of a brain capillary endothelial cell in the lower part of the figure illustrates the current view of the localization of drug efflux transporters at brain capillary endothelial cells that form the BBB. Only those transporters are illustrated for which localization (luminal or abluminal) in brain capillary endothelial cells has been shown. Arrows show proposed direction of the transport. Only efflux transporters that are localized on the apical (luminal) side of the brain capillary endothelium would be in a position to restrict brain uptake of drugs. However, transporters that mediate intracellular uptake at the abluminal membrane may act in concert with efflux transporters at the apical membrane, thereby enhancing extrusion of drugs from the brain. Based on data reviewed recently by Löscher and Potschka (15, 16).

Endothelial cells are attached via periodic junctional proteins, but clefts are maintained by relatively loose attachments between cells. Clefts are normally 6 to 7 nm in size or slightly smaller than an albumin particle. These clefts are more pronounced in the liver such that most plasma substrates freely diffuse from the blood into the liver parenchyma. Plasmalemmal or pinocytic vesicles can be formed at the cell surface, enclosing plasma and substrates, which can then transverse the cytoplasm for delivery and efflux on the opposite side of the cell. Finally, fenestrae can be formed by the endothelial cells, with invaginations creating an increased surface area for substrates to diffuse and be transported into and across endothelial cells. Such fenastrae are especially prominent in the glomerular structure of the kidney (5).

In contrast to the periphery, brain endothelial cells have continuous tight junctions, no fenestrations, and exhibit very low pinocytic activity (6). Brain endothelial cells are also surrounded by a basal membrane and extracellular matrix, as well as pericytes and astrocyte foot processes which further form the BBB and mediate its permeability (Fig. 1B). Astrocyte end-feet cover over 90% of the endothelial cell surface, and the permeability of the BBB is partially under the control of these associated brain

cells. Astroglia can release chemical factors and signals that modulate the permeability of the brain endothelium (7).

In addition to these physical barriers that comprise the BBB, brain capillaries have a high electrical resistance which likely forms a barrier against polar and ionic substances from entering the brain. This resistance is measured at between 1000 and 2000 ohm cm^2, compared with the electrical resistance in peripheral capillaries which typically is measured at 10 ohm cm^2 (8). The cause of this high electrical resistance in the brain capillaries has been thought to be due to differences in protein composition, including the high expression of occludin (9). Table 1 summarizes the differences between the vasculature of the periphery compared with that within the CNS.

The brain capillary network is extensive, and it is estimated that 100 billion capillaries measuring 650 kilometers in length comprise the brain vasculature. The surface area of this capillary endothelium is ~ 20 m^2. The distance from an individual neuron to a brain capillary is between 8 and 20 nm (10). Thus, it is estimated that every neuron is perfused by its own blood vessel, and in turn, substrates are delivered directly to each neuron if the substrate is able to be transported across the BBB (11).

The homeostasis of the brain depends both on the endothelial BBB and on the epithelial blood-cerebrospinal fluid (CSF) barrier located at the choroid plexuses and the outer arachnoid membrane (12, 13). The choroid plexus, which is the main source for CSF production, comprises fenestrated and, therefore, highly permeable capillaries at the blood side that are surrounded by a monolayer of epithelial cells that face the CSF and are joined together by tight junctions (Fig. 2). These tight junctions form the structural basis of the blood-CSF barrier and seal together adjacent polarized epithelial cells (also known as ependymal cells). Thus, once a solute or drug has crossed the capillary wall, it must also penetrate the ependymal cells before entering the CSF (Fig. 2). The surface area of the BBB, however, is much larger than the blood-CSF barrier, estimated to be larger by a magnitude of 5,000-fold (12).

In order for substrates in the systemic circulation to enter the brain parenchyma and cross the BBB, molecules must either passively diffuse or be actively transported across this barrier (Figs. 1 and 3; ref. 13). Mahar Doan et al. (14) analyzed 18 different physiochemical properties of drugs used to treat CNS and non-CNS disease to identify properties that correlated with efficacy in treating the brain. CNS drugs were found to have fewer hydrogen bond donors, fewer positive charges, greater lipophilicity, lower polar surfaces, and reduced flexibility—properties that indicate enhanced membrane permeability. Thus, transcellular passive diffusion is restricted to small (<400 Da), nonpolar, and lipophilic compounds (11). Water-soluble or polar compounds can only penetrate by way of active transport systems across the BBB (15, 16).

Table 1. Comparison of physiologic properties of endothelial cells in the periphery and at the BBB

Property	Periphery	BBB
Intercellular junctions	Loose	Tight
Pinocytic vesicles	Present	Absent
Fenestrae	Present	Absent
Electrical resistance	Low	High

Although it would be expected that lipid-soluble drugs would readily diffuse across the BBB, many of these drugs have been found to have a lower permeability than that predicted by their lipid solubility (17). These drugs, including chemotherapeutic agents, are substrates for drug efflux transporters, which are present in the BBB and blood-CSF barrier, and the activity of these transporters very efficiently removes the drugs from the CNS, thus limiting brain uptake.

Drug Efflux Transporters of the BBB

Researchers have identified numerous efflux transporters that comprise the BBB (Fig. 1B). The most extensively studied is P-glycoprotein, but additional transporters, including members of the multidrug resistance protein (MRP) family as well as members of the organic cation and anion transporter families, have also been identified in the brain endothelium (Fig. 1). The likely role of these transporters in mediating the prevention of drugs, including chemotherapies, from entering the brain has also been fairly well characterized.

P-glycoprotein. P-glycoprotein is encoded by the multidrug resistance gene (*MDR1*) and is also known as ABCB1. P-glycoprotein was initially discovered over 30 years ago as a highly expressed protein in multidrug-resistant tumor cell lines (18). Its encoding gene, *MDR1*, was discovered a decade later (19). Since that time, its role in characterizing a multidrug-resistant phenotype in cancer cells and in tumors *in vivo* has been extensively researched. P-glycoprotein is expressed in numerous tissues, including the gastrointestinal tract (GI), the liver, and the kidneys. It is involved with the absorption from the GI tract as well as excretion into the GI tract of exogenous and endogenous substrates. It is also involved in the renal elimination of substrates from the circulation. P-glycoprotein substrates number in the hundreds if not thousands, and many of the chemotherapeutic agents used in clinical practice are substrates for P-glycoprotein (20, 21). A selection of these agents is listed in Table 2.

P-glycoprotein expression in the brain has been found in numerous species, including humans, primates, rats, mice, and pigs (15). It is principally expressed at the luminal membrane of the brain capillary (Figs. 1 and 4; refs. 22–24). There, it serves as an efflux pump to extrude substrates back into the circulation after they initially diffuse into the endothelial cell. Thus, P-glycoprotein substrates are actively extruded from the brain endothelium back into the circulation, restricting or preventing entry into the brain parenchyma.

P-glycoprotein's role in maintaining the BBB has been extensively studied, both *in vitro* and *in vivo*. For example, *Mdr1* knock-out mice show an increased brain exposure to P-glycoprotein substrates from the circulation compared with wild type (23, 25). In addition, animals treated with inhibitors of P-glycoprotein have been shown to have an increased

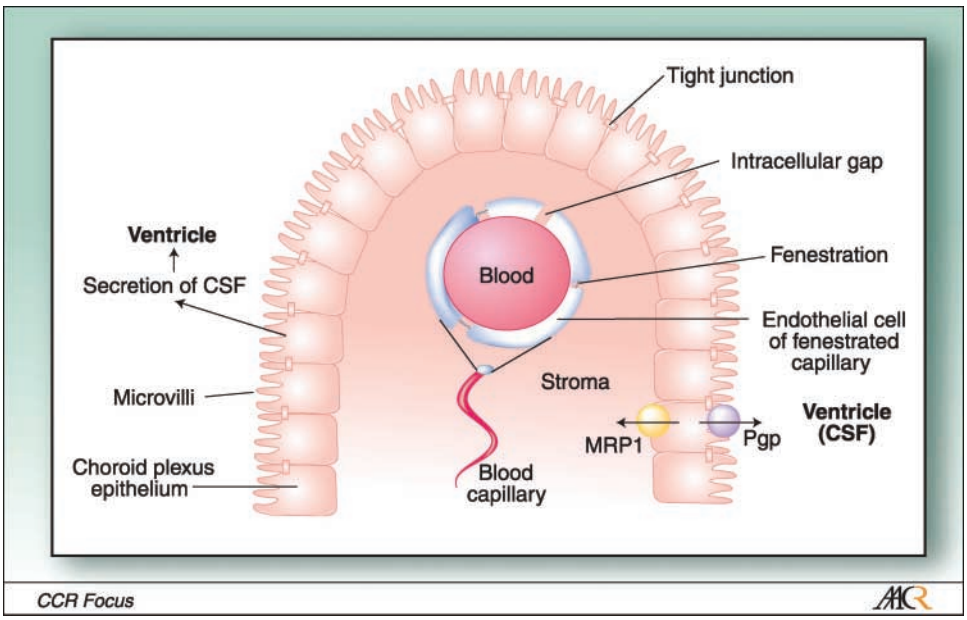

Fig. 2. Schematic representation of the blood-CSF barrier. The capillaries in the choroid plexus allow free movement of molecules via intracellular gaps and fenestrations. The choroid plexus epithelial cells are an integral part of the blood-CSF barrier. The cells are joined together by tight junctions, which limit paracellular flux. The CSF-facing surface of the epithelial cells, which secrete CSF into the ventricles, is increased by the presence of microvilli. The subcellular localization of the multidrug transporters is represented exemplarily in one epithelial cell. With its expression at the basal site of choroid plexus epithelial cells, MRP1 mediates transport out of the CSF into the blood. In contrast, P-glycoprotein seems to be located at the apical site and mediates transport into the CSF, although this view is not unequivocal (see text). It is likely that further multidrug transporters contribute to active transport at the blood-CSF barrier, such as MRP1, MRP4, MRP5, and Oatp3 (15).

exposure in the CNS from P-glycoprotein substrates (25–30). For example, Wang et al. (28) administered rhodamine 123, a fluorescent P-glycoprotein substrate, with the P-glycoprotein inhibitor cyclosporine A and found increased brain fluorescence in a rat model. Using *in vivo* models, increased brain penetration of chemotherapeutic agents has been shown using P-glycoprotein inhibition and the agents vincristine (29), paclitaxel (30), and daunorubicin (31), among others. Mice with a xenograft glioblastoma tumor given paclitaxel with the P-glycoprotein inhibitor PSC 833 exhibited increased brain concentration of paclitaxel and improved tumor response compared with animals given paclitaxel alone (32). Similar results were found using the P-glycoprotein inhibitor zosuquidar and paclitaxel (33). These animal studies on increased brain penetration of drugs after P-glycoprotein inhibition have been confirmed in a human study using [^{11}C]-verapamil, a P-glycoprotein substrate. Sasongko et al. (34) used the P-glycoprotein inhibitor cyclosporine A and found increased brain accumulation of labeled verapamil as measured by positron emission tomography imaging in patients given cyclosporine.

In the blood-CSF barrier, P-glycoprotein is expressed in the proximity of the apical membrane of the choroid plexus (ref. 35; Fig. 2). In this location, it likely transports substrates into the CSF from the endothelium and the brain parenchyma. This activity reduces the concentration of P-glycoprotein substrates in the choroid plexus and transports them into the larger volume of the CSF. This location of P-glycoprotein in the choroid epithelium has been somewhat debated because at least one study found that P-glycoprotein was expressed in vesicles adjacent to the apical membrane in the choroid epithelium rather than in the membrane itself (36). P-glycoprotein may serve there as a transporter that effluxes drugs into vesicles. These vesicles may, in turn, be inserted into the apical membrane for substrate exocytosis from the cell into the CSF.

Multidrug resistance–associated proteins. In addition to P-glycoprotein, numerous other transporters are involved in forming the BBB. These include multidrug resistance protein 1, or MRP1, as well as MRP2 through MRP9, all members of the ABCC family of transporters. In brain capillary endothelial cells forming the BBB, recent studies have reported a predominantly apical plasma membrane distribution for MRP1, MRP2, and MRP5 and an almost equal distribution of MRP4 on the apical and basolateral plasma membrane (ref. 37; Fig. 1). Varying expression of ABCC transporters have been found in the human BBB, as well as the BBB seen in other species.

The role of MRP1 in contributing to the BBB has been confirmed with the development of an *Mrp1*–knock-out mouse. Substrates, including the MRP1 substrate etoposide, were found at higher levels in the brains of knock-out animals compared with wild-type mice (38, 39). Further evidence was found by Sun et al. (40), who administered the MRP inhibitor probenecid with fluorescein, a known substrate. Coadministration of substrate and inhibitor led to a 2-fold increase in the CNS concentration of fluorescein. Substrates for and inhibitors of members of the ABCC family are listed in Table 2.

In the choroid plexus, the ABCC family member MRP1 is expressed on the basolateral side of the endothelium, mediating transport into the blood from the CSF (35). This is in contrast to the role of P-glycoprotein at this location (Fig. 2). It has been suggested that these two transporters might coordinate the absorption and secretion of compounds across the CNS at the blood-CSF barrier.

Breast cancer–resistant protein (ABCG2). The transporter ABCG2, initially named the breast cancer–resistant protein (BCRP), was first discovered in a chemotherapy-resistant breast cancer cell line (41). Since its discovery, it has been extensively studied and found to play a critical role in various physiologic processes. The transporter is expressed on the luminal surface of the GI tract, mediating absorption of its substrates. It also lines

the bile canniculi as well as renal tubules, mediating efflux of substrates into the bile and urine. It is expressed in the placenta, forming part of the maternal/fetal barrier, and it contributes to the germ cell–blood barrier by its expression in the testes and the ovary (42). In the brain, ABCG2 has been detected in capillary endothelial cells in humans as well as in other species. Like P-glycoprotein, it is expressed mainly on the luminal surface (refs. 43, 44; Fig. 1 and Fig. 4). Based on mRNA expression, ABCG2 may be even more strongly expressed than P-glycoprotein or MRP1 (44). Substrates and inhibitors of ABCG2 are listed in Table 2.

Breedveld et al. (45) studied the brain penetration of imatinib, a substrate for ABCG2, in *Bcrp1* knock-out mice, and found a 2.5-fold increase in brain concentration in knock-out animals compared with wild-type animals. In addition, these researchers administered imatinib with elacridar, an inhibitor of ABCG2, to wild-type animals and found an increased brain penetration by 4.2-fold. Following administration of an ABCG2 inhibitor, GF120918, to *Mdr1* knock-out mice who lacked expression of P-glycoprotein, Cisternino et al. (46) found increased brain uptake of the ABCG2 substrates prazosin and mitoxantrone.

Organic anion and cation transporters. The more recently discovered organic anion and cation transporter families have also been found in brain endothelium and likely play a role in forming the BBB. The organic anion transporters (OAT) OAT1 and OAT2 are both transporters that line the biliary canniculi and renal tubules and thus serve in the excretion of organic anions into the bile and urine. As opposed to ABC transporters such as P-glycoprotein, which require ATP for active transport, organic anion and cation transporters typically exchange anions and cations across concentration gradients from the blood to the brain or in reverse. Thus, drug transport into and out of the brain will depend on the ionic or drug gradients (15).

The exact localization of transporters from the OAT family as well as the organic anion–transporting polypeptide (OATP) family, another subfamily of anion transporters, has not been clearly identified in the brain capillary endothelium. In the rat, Oatp2 is located in both the apical and basolateral membranes of brain endothelium (Fig. 1; ref. 47). In humans, OAT3 has been found to be predominantly expressed at the basolateral membrane (Fig. 1; ref. 47). OATP-A is also expressed in brain endothelia, and it may also form part of the BBB (48). The cation transporter OCT2 is expressed in the choroid plexus and localized subcellularly in cytosolic vesicles or in the apical membrane, serving a similar role as P-glycoprotein in the choroid plexus. OAT1 has been found to be expressed in the brush border of the choroid plexus.

Relatively few studies have examined the role of OATs and OATPs in forming the BBB, although in the mouse model, at least Oatp3 has been shown to play a significant role (24). These studies are difficult to perform given the overlapping substrate specificity of ABC transporters and OATs/OATPs. The exact role these transporters play in mediating systemic pharmacokinetics, including renal elimination, of cancer and

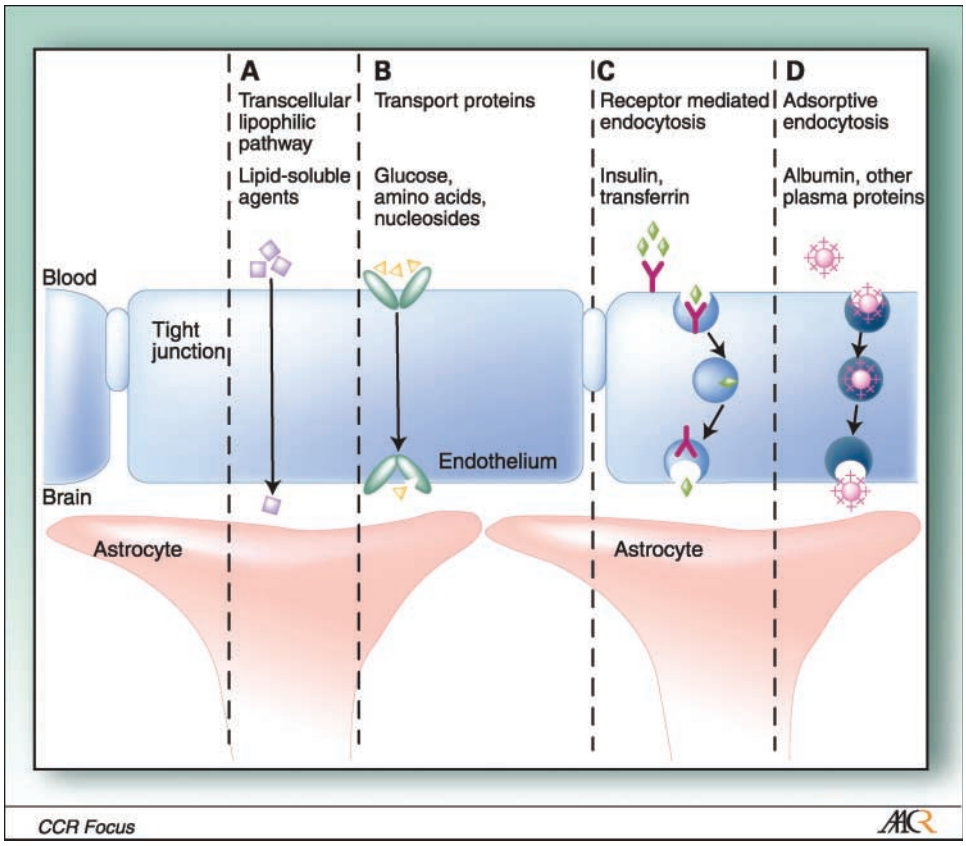

Fig. 3. Schematic representation of mechanisms available for endogenous substrates to cross the BBB. *A,* small, lipid-soluble substrates are able to diffuse across the membrane, although are subject to efflux back into the circulation by transporters as discussed in the text. *B,* small endogenous molecules, including amino acids, nucleosides, and glucose are transported across the BBB by transport proteins. *C,* receptors for endogenous larger molecules, including insulin and transferrin, are recognized by receptors on the luminal side of the endothelium, are endocytosed, and transported across the cell for release into the brain parenchyma. *D,* endogenous large plasma proteins including albumin are transported across the BBB by adsorptive-mediated endocytosis. Modified with permission from ref. (7).

Table 2. Drug transporters putatively involved in forming the BBB, as well as chemotherapy agents that are substrates for each transporter, and compounds used as potential inhibitors of transporter function

Transporter	HUGO name	Substrates	Inhibitors
P-glycoprotein	ABCB1	Doxorubicin, daunorubicin, docetaxel, paclitaxel, epirubicin, idarubicin, vinblastine, vincristine, etoposide	Verapamil, cyclosporine A, quinidine, PSC 833 (valspodar), GF120918 (elacridar), VX-710 (biricodar), LY335979 (zosuquidar), XR9576 (tariquidar)
MRP1	ABCC1	Etoposide, teniposide, daunorubicin, doxorubicin, epirubicin, melphalan, vincristine, vinblastine	Probenecid, sulfinpyrazone, MK-571, some P-glycoprotein inhibitors (e.g., cyclosporin A, verapamil, PSC 833)
MRP2	ABCC2	Similar to MRP1	Probenecid, MK-571, leukotriene C4
MRP3	ABCC3	Similar to MRP1	Sulfinpyrazone, indomethacin, probenecid
MRP4	ABCC4	Methotrexate, 6-mercaptopurine, thioguianine	Probenecid
MRP5	ABCC5	6-Mercaptopurine, thioguanine	Probenecid, sildenafil
MRP6	ABCC6	Actinomycin D, cisplatin, daunorubicin, doxorubicin, etoposide	Probenecid, indomethacin
BCRP	ABCG2	Mitoxantrone, methotrexate, SN-38, topotecan, imatinib, erlotinib, gefitinib	GF120918, fumitremorgin C

other therapeutic drugs is an active area of research, and their role in forming the BBB will hopefully be elucidated in the years ahead.

The BBB in Primary or Metastatic Brain Tumors

A number of investigators have explored the integrity of the BBB in both primary and metastatic cancerous tumors. In these studies, alterations in brain endothelial cells have been described which differentiate these blood vessels from normal brain vasculature (49). These alterations include a compromised tight junction structure and increases in the perivascular space (50). In addition, in the blood vessels within these tumors, fenestrations can be found that mirror the vasculature in the periphery. Finally, an increased number and activity of pinocytic vacuoles are present (51). Thus, it seems that these vessels may reflect those of the tissue of tumor origin rather than CNS endothelial cells.

The expression of transporters is also altered in the endothelial cells that form the vasculature around tumors compared with normal brain vasculature. Regina et. al. (52) found that the expression level of P-glycoprotein in blood vessels supplying melanoma CNS metastases was only 5% of that seen in normal brain tissue. They also reported that the vasculature around CNS lung metastases had only 40% of the P-glycoprotein expression found in normal brain vasculature. Conflicting evidence exists on the level of P-glycoprotein in the vasculature of malignant gliomas, with Becker et al. (53) finding diminished expression compared with normal brain vasculature, whereas Toth et al. (54) found no difference between the P-glycoprotein expression in tumor vasculature and normal tissue.

Haga et. al. (55) investigated the expression level of members of the MRP family. They found no difference in the expression level of P-glycoprotein and MRP2 between normal brain and malignant glioma cells. However, they did find increased expression of MRP1 and MRP3 in the endothelial cells forming the vasculature around tumor sites.

Thus, the BBB is less intact in primary and metastatic brain tumors compared with the normal brain vasculature. Although there is a spectrum of barrier integrity and permeability, in general, the BBB at sites of tumor seems to have an increased level of permeability. This has led some to describe the tumor capillary bed as a "blood-tumor barrier" rather than a BBB.

Radiation and the BBB

Radiation is standard therapy for most primary and metastatic CNS malignancies, as discussed at length elsewhere in this Focus section. Investigators have found that radiation can disrupt the BBB, which could enable more successful delivery of chemotherapy agents. Rubin et al. (56) irradiated rat brains with 60 Gy and found BBB disruption and vascular leak at 2 weeks postexposure. Reinhold et al. (57) found more diffuse changes in rat brains when using more therapeutically relevant dosages of 20 to 25 Gy, including dilation of the blood vessel lumen, thickening of the blood vessel wall, enlargement of endothelial cell nuclei, and hypertrophy of adjacent astrocytes. Mima et al. (58) found that in rat brains exposed to 25 Gy of radiation the P-glycoprotein expression was reduced to 60% of that seen in control animals. More recently, researchers have used focused ultrasound to successfully disrupt the BBB in a rabbit animal model (59).

Chemotherapy Agents and the BBB

Given that the BBB is an efficient barrier against entry of many of our commonly used chemotherapy agents, but also that the BBB is disrupted at the site of metastatic disease, what has been the clinical experience with treating brain malignancy compared with treating primary tumors in the periphery? Very few studies in patients with metastatic disease to the brain report response rates separately for brain and extracranial lesions. In general, clinical trials that report results that allow comparison have shown that brain metastases respond as well to chemotherapy treatment as do extracranial primary and metastatic tumors. For example, Rosner et al. (60)

reported on the treatment of 100 consecutive breast cancer patients with symptomatic brain metastases treated with various combinations of chemotherapy and found that 50% of patients had objective responses in their brain disease, a response rate similar to the response rate in peripheral lesions. Lee et al. (61) treated 14 patients with brain metastases from small cell lung cancer with cyclophosphamide, doxorubicin, vincristine, and etoposide. Nine of 11 (82%) patients

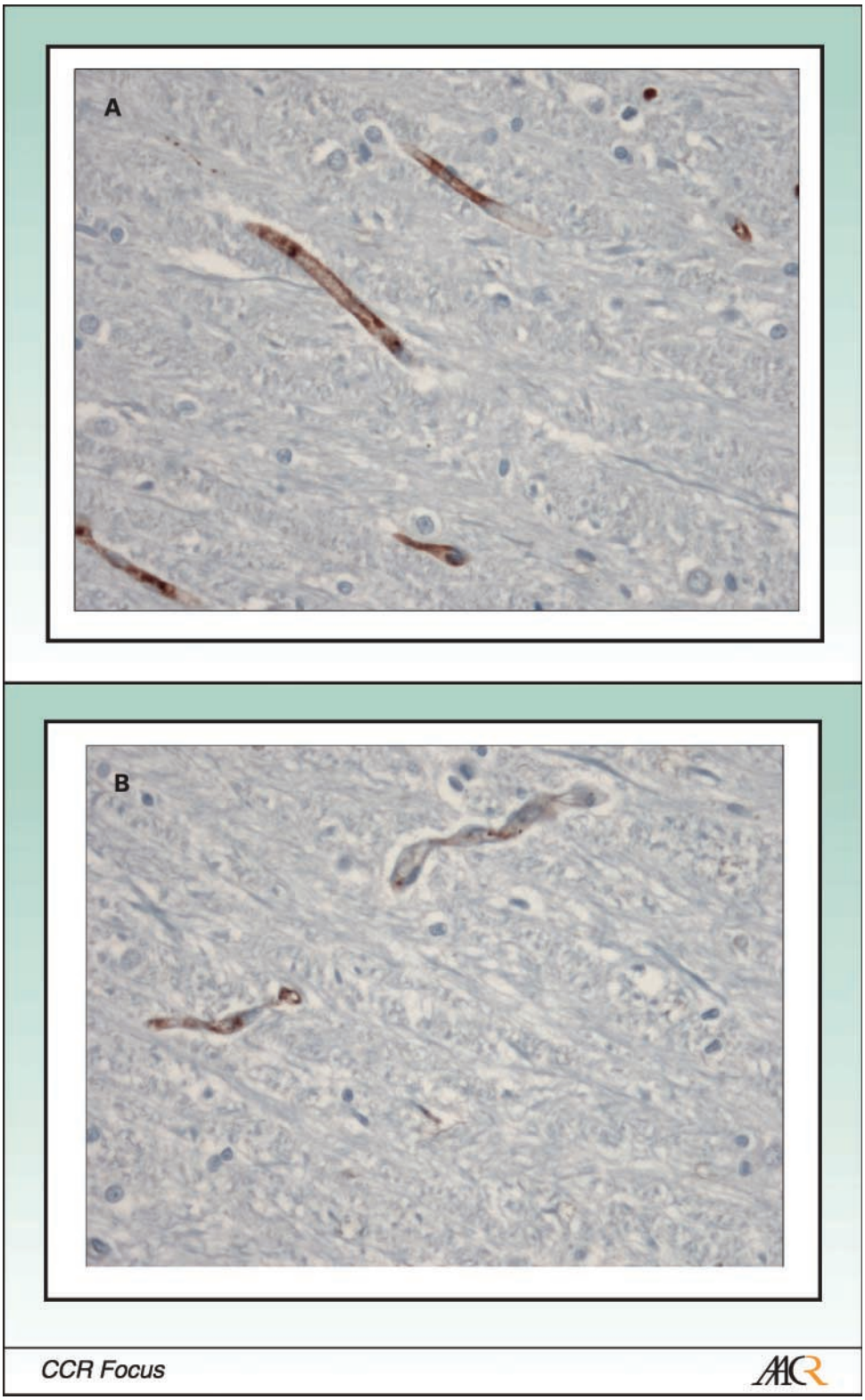

Fig. 4. Immunohistochemistry staining for (*A*) ABCG2 using the 1:50 BXP-21 antibody (Kamiya Biomedical Company) and for (*B*) P-glycoprotein using the 1:10 C219 antibody (Signet Laboratories). Staining for both proteins is demonstrated in the endothelial cells in capillaries in the midbrain. Magnification, 120×, courtesy of Patty Fetsch and Armando Filie, National Cancer Institute.

Table 3. Drug levels in brain metastases and neighboring tissues, measured at time of resection or at autopsy

Drug	Tumor level: necrotic lesion	Tumor level: viable lesion	Neighboring normal brain level
Etoposide (63)	5.9 μg/g	3.4 μg/g	1.4 μg/g adjacent, 0.1 μg/g 2 cm distance
Cisplatin (64)			
20 to 25 mg/m^2	Not reported	1.29 μg/g	0.25 to 0.65 μg/g, 2 to 5 cm
60 to 100 mg/m^2		2.97 μg/g	0.7 to 1.11 μg/g, 2 to 5 cm
Vinorelbine (65)	Not reported	68 ng/g	22 ng/g adjacent, 5 ng/g >4 cm
Mitoxantrone (66)	15 to 322 ng/g	25 to 29 ng/g	Not reported

evaluated had responses in their brain lesions, whereas 9 of 12 evaluated patients had responses in their extracranial lesions. Postmus et al. (62) treated patients with small cell lung cancer and brain metastases with teniposide with or without whole brain radiotherapy and found that the clinical responses in brain lesions were similar to those in metastases outside of the brain using this drug. Fujita et al. (63) treated 30 patients with non–small cell lung cancer with cisplatin, ifosfamide, and irinotecan with rhG-CSF support. The response rate was 50% in brain lesions and 62% in extracranial primary or metastatic lesions. Bernardo et al. (64) treated 22 patients with non–small cell lung cancer with vinorelbine, gemcitabine, and carboplatin and also found that brain and extracranial response rates were similar. Finally, Korfel et al. (65) treated 22 patients with symptomatic small cell lung cancer metastatic lesions in the brain with topotecan after whole brain radiation. The response rate in brain lesions was 33%, whereas the response rate in primary or systemic metastatic disease sites was 29%.

In fact, radiographically and/or symptomatic brain metastatic disease does respond to systemic chemotherapy. As reviewed by Tosoni et al. (4), response rates to various chemotherapy agents or combinations of agents by primary disease sites are as follows: non–small cell lung cancer, 0% to 82%; small cell lung cancer, 0% to 92%; breast cancer, 0% to 58%; and melanoma, 0% to 50%.

So how do we explain the apparent contradiction that radiographically evident brain metastases respond to therapy, while at the same time brain metastases is a known poor and independent prognostic factor for survival in diseases such as lung and breast cancer, and that the incidence of brain metastases is going up as our chemotherapy agents and combinations are improving in their efficacy? The answer lies in what is known about how the BBB is disrupted at the site of significant brain disease. At these sites, the 'tumor-blood barrier' is greatly impaired in terms of transporter expression and function, as well as in terms of the permeability of the endothelium. This allows sufficient systemically delivered chemotherapy to reach the tumor and effect a response. However, this is only at larger brain lesions. In smaller aggregates of metastatic tumor cells, the disruption of the BBB is less significant. Therefore, less drug reaches these so-called micrometastases, and they are allowed to continue to grow, develop neovasculature structures, and ultimately reach a clinically significant size. This theory is partially confirmed by the fact that as our technical ability to radiographically detect brain metastases has improved from the use of first CT scans and now magnetic resonance imaging, more patients are now found at the time of evaluation to have brain involvement with small lesions in addition to larger and symptomatic lesions.

This theory that an intact BBB protects smaller nests of tumor cells from systemic chemotherapy would be confirmed if cytotoxic drug levels in the brain could be compared between sites of larger metastatic lesions and nearby but apparently normal brain parenchyma. Remarkably, very few studies have evaluated drug levels in brain tissues to help answer this question. This is due in part to the difficulty in sampling drug levels in any tissue, especially within the cranium. However, a number of studies done in the 1980s investigated drug levels in brain tissues of chemotherapy agents including etoposide (66), cisplatin (67), vinorelbine (68), and mitoxantrone (69), either at time of resection or at autopsy, and found higher levels at sites of larger tumors than in neighboring tissues. Table 3 lists the results from these studies. Interestingly, the highest concentrations were typically found in central areas of tumor necrosis. Drug levels found in brain tumors were lower than those found in extracranial tumors, but in most cases, the levels were thought to be sufficiently high compared with *in vitro* assays to induce cytotoxic activity.

Increasing Drug Delivery to Brain Tumors

Given the need to better deliver cytotoxic therapy to brain lesions, both larger lesions as well as micrometastases that may evade typical systemic chemotherapy, strategies to enhance drug delivery have been pursued. These methods to increase drug delivery to the brain have, in general, followed three approaches. The first, and one in common clinical use, is the administration of chemotherapy agents directly into the CNS. This includes the use of Ommaya reservoirs, intrathecal injections (70), intra-arterial injections (71, 72), or high-dose systemic therapy with agents such as methotrexate (73). Although these approaches have found some success, toxicities can be significant. A second strategy is to deliver chemotherapy drugs with an agent that inhibits the drug transporter(s) of the BBB. A final strategy would be to disrupt the BBB followed by the administration of chemotherapy.

Inhibiting BBB Transporters

P-glycoprotein inhibitors. The development of agents to inhibit P-glycoprotein at the cellular level, and thus, increase

intracellular concentrations of toxic chemotherapy agents in resistant tumors, has been a research approach pursued by a large number of basic and clinical researchers over the past two decades (74). Inhibitors tested clinically include verapamil, quinidine, cyclosporine, PSC-833 (valspodar), GF120918 (elacridar), and XR9576 (tariquidar). Clinical trials in both solid and hematologic malignancies testing P-glycoprotein inhibitors with cytotoxic P-glycoprotein substrates to overcome cancer cell resistance have been disappointing. Promising phase II trials were followed by negative phase III trials, at times with trials being stopped early due to unacceptable toxicities.

These negative results have put in doubt the strategy of overcoming cellular drug resistance by the use of P-glycoprotein inhibitors. However, the potential role of P-glycoprotein inhibitors in overcoming the BBB is still an open question. In animal models, the administration of P-glycoprotein inhibitors has been found to increase intracranial concentrations of chemotherapy agents. In mice given PSC-833 with paclitaxel, increased concentrations of paclitaxel were found in the brain, and these increased concentrations led to a higher tumor response in these mice (32). Additional studies using animal models have found increased concentrations of vinblastine as well as morphine-6-glucuronide when coadministered with PSC-833 and GF 120918 (25). Further evaluation of the use of these inhibitors, and their potential role in inhibiting P-glycoprotein at the BBB and, thus, increasing drug levels within the CNS, is warranted.

The other BBB transporters. Inhibiting other transporters involved in forming the BBB has also been studied, although not pursued clinically to the extent seen with P-glycoprotein. Nonselective inhibitors of MRP-1, including probenecid, indomethacin, and VX-710 (biricodar), which also inhibits P-glycoprotein, have been tested preclinically. The administration of VX-710 with substrates of MRP-1, including vincristine, doxorubicin, and etoposide, in neuroblastoma cell lines has been shown to increase the cellular sensitivity to chemotherapy (75). Given the overlap in chemotherapy agents that are substrates for MRP-1 as well as other transporters, the clinical research of MRP inhibition has not been actively pursued.

The inhibition of ABCG2 (BCRP) has also been looked at in preclinical models. Agents including fumitremorgin C and GF 120918, which acts as an ABCG2 inhibitor as well as a P-glycoprotein inhibitor, have been administered with chemotherapy agents in preclinical models. Treating mice with GF120918 and the ABCG2 substrates mitoxantrone and imatinib led to higher CNS penetrance of drug in these models (45, 76). As with MRP1, it is unclear whether further clinical development of ABCG2 inhibitors will be pursued for systemic disease, but research into their use as part of a strategy of increasing drug levels within the CNS is certainly warranted.

Disrupting the BBB

In addition to administering inhibitors of drug transporters, another strategy to increase the CNS penetration of chemotherapy agents has been the disruption of the BBB using hypertonic solutions such as mannitol (77). Another agent that has been found to disrupt the BBB is RMP-7, a synthetic analogue of the peptide bradykinin with specificity for the bradykinin-P2 receptor. Bradykinin helps modulate the tight junctions seen in brain endothelial cells. The administration of RMP-7 has been found to disrupt these tight junctions and increase the permeability in brain endothelial cells (78). Using preclinical models, RMP-7 has been found to increase the brain concentration of carboplatin when intracarotid or i.v. administration of this drug was given along with RMP-7 (79). Although some problems were found in these animal models, including tachyphylaxis and a rapid restoration of the BBB within an hour, these studies were successful enough to pursue studies in humans in phase I and phase II clinical trials. Unfortunately, in two phase II trials testing this combination in childhood as well as recurrent adult primary CNS malignancies, activity was not observed (80, 81). The researchers wondered whether a higher dose of RMP-7 may have been necessary to have the desired effect within the CNS. Further studies are necessary to determine whether this or a similar vehicle is advantageous for delivering chemotherapy into the CNS.

New Drug Class Formulations for Crossing the BBB

Nanoparticles. One strategy for delivering drugs across the BBB has been encasing the compounds within nanoparticles (Fig. 2). For example, researchers have encapsulated chemotherapy agents and other drugs in 250-nm-diameter nanoparticles using poly(butyl)cyanoacrylate (PBCA; ref. 36). These small PBCA nanoparticles are coated with Tween-80 nanoparticles, which apparently bind apolipoprotein E to the particles. This coating of the small lipoprotein with apolipoprotein E seems to mask the nanoparticle and make it appear as a low-density lipoprotein. These nanoparticles are endocytosed by the endothelium of the BBB, allowing entry of the particle into the endothelial cell. The drug can then diffuse or be effluxed into the brain parenchyma. Numerous compounds have been encapsulated this way, including doxorubicin (82). Using an animal model, researchers found that brain concentration of doxorubicin were higher by more than a log value when delivered as a nanoparticle. Fabel et al. (83) treated patients with high-grade malignant gliomas with liposomal doxorubicin and found moderate activity and improved overall survival when compared with past trials using other possible therapies. Hau et al. (84) used a pegylated liposomal formulation of doxorubicin in patients with recurrent high-grade glioma and also found it to be moderately effective and well tolerated. Further clinical trials using these and additional nanoparticle formulations of cytotoxic agents are warranted.

Immunoliposomes. Another targeting approach to delivering drugs across the BBB is by tagging or attaching liposome-containing drugs with an antibody that recognizes receptors along the endothelium. Endogenous large-molecule peptides such as transferrin, insulin, and leptin cross the BBB via attachment to such receptors and cross via receptor-mediated transport (ref. 11; Fig. 3). Monoclonal antibodies attached

to liposome-containing drugs can recognize these receptors, be recognized as ligands, and be endocytosed. The monoclonal antibody OX26, which recognizes the transferrin receptor, was attached to a liposome-containing digoxin, allowing digoxin to traverse the BBB in animal models. Huwyler et al. (85) used the OX26 immunoliposome to transport daunorubicin using an animal *in vivo* model and found increased brain delivery leading to a higher concentration compared with administering drug without this vehicle by a factor of 4 log values. Monoclonal antibodies directed against the insulin receptors have also been developed for this same drug-delivery purpose.

Peptide vectors. Linking compounds with peptide vectors that enable passage across the BBB is another potential strategy. Mazel et al. (86) linked doxorubicin to two peptide vectors in which tertiary structures allow increased penetration across biological membranes. When administered to *Mdr1* knock-out and wild-type mice, this formulation of doxorubicin was able to bypass P-glycoprotein on the luminal side of the BBB and have comparable drug delivery in wild-type mice compared with knock-out animals.

Carrier-mediated transport through the BBB. Another potential strategy is to develop agents that are substrates for the influx transporters that mediate endogenous substrate transport from the circulation into the brain parenchyma. These influx transporters that line the endothelium, both on the luminal surface as well as the basolateral surface, could be used to mediate the delivery of drugs directly into the CNS. For example, the drug L-3,4-dihydroxy-L-phenylalanine (L-DOPA) is a precursor of dopamine. Dopamine is water soluble and does not cross the BBB. However, L-DOPA is transported via the L-type amino acid transporter 1 (LAT 1) and, thus, is taken up from the circulation in the brain endothelium directly for delivery to the brain parenchyma. Once transported by the LAT 1 transporter, L-DOPA is converted to dopamine in the brain to exert its therapeutic effect. Other drugs thought to be transported by LAT 1 include melphalan and gabapentin (13). Designing drugs such that they are substrates for these transporters is a potentially important strategy to circumvent the efflux capacity of the BBB and increase drug delivery.

Conclusion

The past decade has produced significant research into the physiology of the BBB, including the role of transporters mediating this protective barrier. This barrier helps explain the lack of efficacy in using pharmaceuticals to treat CNS disease, ranging from epilepsy to malignancy (15). It was once thought that a single transporter, P-glycoprotein, mediated much of this barrier. It is now understood that numerous transporters mediating exogenous and endogenous transport line the BBB as well as the blood-CSF barrier. These transporters are not just efflux proteins expressed on the luminal surface, but instead comprise a complicated influx and efflux dynamic system. This system enables the absorption of some compounds from the circulation into the brain, but also mediates the efflux of drugs, endogenous substrates, and toxins back into the circulation.

In the case of malignancy, in both tumors that arise primarily in the CNS as well as metastatic disease from distant sites, the BBB is partially compromised and, thus, enables some drug to be delivered to the tumor site. However, this barrier is sufficiently intact such that many of our common chemotherapy agents are not as effective when treating CNS disease, especially micrometastatic disease, as they are when they treat tumors in the periphery.

A better understanding of this physiology has led to a number of strategies to enhance delivery of chemotherapy agents to brain malignancies. These recent discoveries and proposed new strategies present exciting opportunities in pharmaceutical and oncological research. Although much of this preclinical and clinical research has been encouraging, much still needs to be done. In the years ahead, these discoveries will hopefully lead toward successful treatment strategies to treat patients with primary and metastatic brain malignancies.

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