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Review

Functional Expression and Localization of P-glycoprotein in the Central Nervous System: Relevance to the Pathogenesis and Treatment of Neurological Disorders

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The expression of membrane drug transport systems in the central nervous system plays an important role in the brain disposition and efficacy of many pharmacological agents used in the treatment of neurological disorders such as neoplasia, epilepsy, and HIV-associated dementia. Of particular interest is P-glycoprotein, a membrane-associated, energy-dependent, efflux transporter that confers the multidrug resistance phenotype to many cells by extruding a broad range of xenobiotics from the cell, resulting in poor clinical outcomes. In addition, the expression pattern of P-glycoprotein has recently been suggested to play a key role in the etiology and pathogenesis of certain diseases such as Alzheimer's and Parkinson's diseases. This review will focus on the cellular localization, molecular expression, and functional activity of P-glycoprotein in several compartments of the central nervous system and address its relevance in the pathogenesis and pharmacological treatment of neurological disorders.

KEY WORDS: brain; neurological diseases; P-glycoprotein; transporter.

INTRODUCTION

Membrane drug transporters and their modulatory effects on drug therapy is an area of research that is growing in interest and momentum, particularly in the field of neuropharmacology. P-glycoprotein (P-gp), an efflux drug transporter, has received particular attention during the years after this protein was discovered to play a key role in the multidrug resistance (MDR) phenotype of some cancer cells. In addition to being expressed in cancer cells, P-gp has been localized to many normal tissues of the body such as the intestine, kidney, blood-brain barrier (BBB), blood-cerebrospinal fluid (BCSF) barrier, blood-testis barrier, pancreas, and in peripheral immune cells. Although no definite physiological role has yet been determined for P-gp, the polarized expression of this protein at various barriers suggests that it may serve to protect the cells from the accumulation of toxic xenobiotics. *In vivo* and *in vitro* studies suggest that the efflux of many pharmacological agents out of cells is mediated by P-gp. More specifically, studies show P-gp to be expressed and functionally active in specific areas of the brain such as the BBB, BCSF barrier, astrocytes, and microglia. In the clinical setting, some brain pathologies (brain neoplasia, epilepsy, HIV-associated dementia) that have shown either poor initial response or acquired resistance to drug therapy express up-regulated levels of P-gp along their cell plasma membranes.

With the discovery of the role of P-gp in drug resistance and disease pathology, much effort has been invested into developing compounds that inhibit P-gp function as adjuncts to therapy. Unfortunately, the clinical efficacy of the first and second-generation P-gp inhibitors has proven to be quite disappointing to say the least, primarily due to their high toxicity profiles (1–3). In addition, the expression of several other families of membrane drug transporters with overlapping substrate specificities poses a significant problem. The more recently developed third generation of P-gp inhibitors show greater specifity and lower toxicity profiles than their predecessors. Their safety and efficacy, however, remains to be demonstrated in clinical trials (4–7). It is believed that these inhibitors may be valuable agents in circumventing the current problems associated with drug resistance in the treatment of brain neoplasias, epilepsy, and HIV-associated dementia. In the case of Alzheimer's and Parkinson's diseases, it seems as though the expression levels of P-gp may be involved in the etiology and pathognesis of these conditions.

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ABBREVIATIONS: AD, Alzheimer's disease; AEDs, antiepileptic drugs; BBB, blood-brain barrier; BCSF, blood-cerebrospinal fluid; BCRP, breast cancer resistance protein; BMRP, brain multidrug resistance protein; CNS, central nervous system; CP, choroid plexus; CR-3, complement 3 receptor; CSF, cerebrospinal fluid; GFAP, glial fibrillary acidic protein; HAART, highly active antiretroviral therapy; HAD, HIV-associated dementia; HIV, human immunodeficiency virus; MDR, multidrug resistance; MRP, multidrug resistance protein; PBMC, peripheral blood mononuclear cell; PD, Parkinson's disease; P-gp, P-glycoprotein; PI, protease inhibitor; RT-PCR, reverse transcriptase polymerase chain reaction.

FUNCTIONAL AND MORPHOLOGICAL CHARACTERISTICS OF THE BRAIN VASCULAR BARRIERS AND BRAIN PARENCHYMA

Brain Vascular Barriers (Blood-Brain Barrier, Blood-Cerebrospinal Fluid Barrier)

The CNS is a delicate microenvironment containing about 10^{11} neurons and at least 1000-fold more dendrites where various interactions and electrical impulses are produced every second. These multifaceted interactions result in reactions ranging from autonomic responses to voluntary movements to higher cognitive functions of memory and thought (8). Two physiological barriers that separate it from the rest of the body, the BBB and the BCSF barrier, keep this highly complex environment under strict homeostatic control.

The BBB is composed of brain endothelial cells that line the cerebral vasculature (Fig. 1a). Tight junctions seal these cerebral endothelial cells together to prevent the bulk flow of water and solutes (9) (Fig. 1b). In fact, brain capillaries are about 50–100 times tighter than peripheral microvessels (10). Furthermore, the physical barrier properties of the BBB also include the absence of intercellular clefts, lack of fenestrations, minor pinocytic activity, and a high transendothelial electrical resistance of about 1500–2000 Ohm cm^2 (11,12). The outer surface of the endothelium is surrounded by the end feet of neighboring astrocytes and pericytes (13). In addition to the physical barrier properties, the cerebral endothelial cells display an asymmetric array of receptors, ion channels, and transporters that function in a dynamic manner to regulate the influx and efflux of molecules, some of which are biologically important and others potentially toxic to the brain.

There are many pathways of drug transport across the various cell membranes of the brain (14,15) (Table I). The mechanism of transport will depend on the physicochemical characteristics of the drug as well as the barrier properties of the brain microvasculature and choroid plexus. One transport pathway is passive permeability of water-soluble drugs across aqueous channels. This method of transport largely depends on the molecular size of the drug. Generally, transport and permeability of compounds with a molecular weight larger than 150–200 is restricted from crossing due to the small openings of aqueous channels of the cell membrane. Examples of highly water-soluble drugs that cross by simple passive diffusion via aqueous channels are acetylsalicylic acid, nicotine, caffeine, and vitamin C (14). Some lipid-soluble drugs cross cell membranes by passive transport between the lipid molecules of the cell membrane. This is largely determined by the following physicochemical characteristics of the drug itself: concentration or dose, oil/water partition coefficient, concentration of proton, and surface area for drug diffusion (14,15). Specialized vesicles that are highly expressed along the cerebral endothelial cell (16) carry out general endocytotic and transcytotic processes. Caveolae are specialized microdomains of the endothelial cell plasmalemma that are rich in cholesterol and exhibit various surface markers such as caveolins, Ca2+-ATPase, alkaline phosphatase, and 5′-nucleotidase that distinguish it from the plasmalemmal portion of the cells (16,17). Functionally, caveolae have been implicated in various transport mechanisms across the BBB such as transcytosis (16), potocytosis (18), and endocytosis

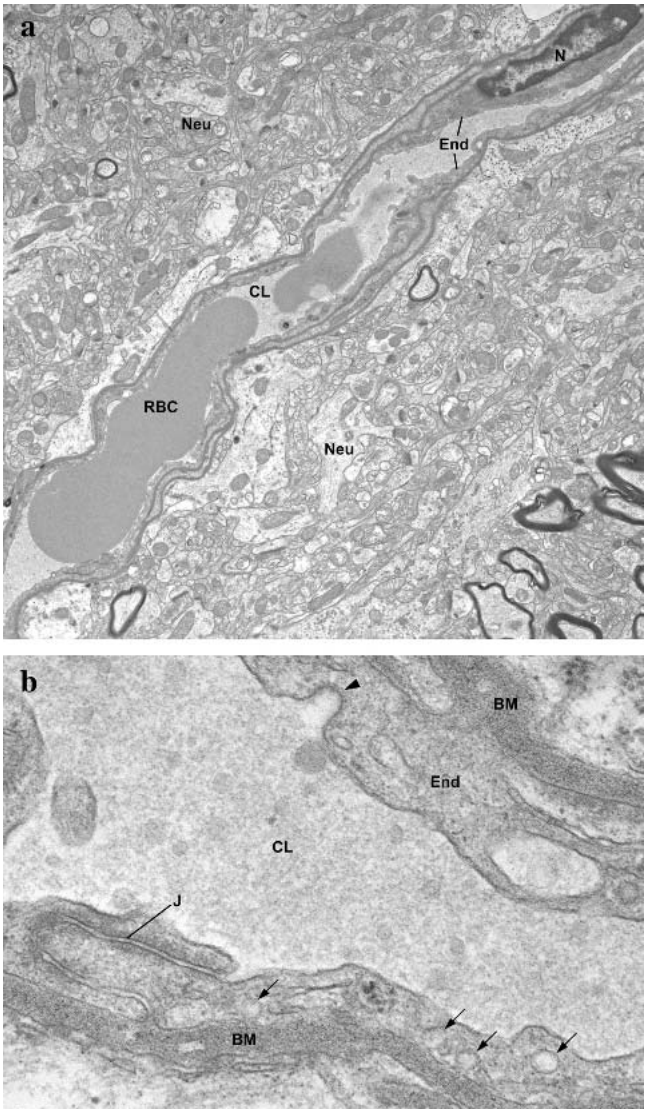

Fig. 1. (a) Ultrastructural features of the blood-brain barrier at the electron microscope level. A capillary blood vessel in longitudinal section, with its capillary lumen (CL) and endothelial cells (End), is seen among the neural tissue (Neu). Within the capillary we can distinguish the profile of two red blood cells (RBC) (magnification, ×4,000). (b) High magnification of the blood capillary. The endothelial cells (End) demonstrate a dense cytoplasm with smooth-membrane caveolae (arrows) as well as a clathrine-coated pit (arrowhead). J, junctional cleft between two endothelial cells. BM, basement membrane. CL, capillary lumen (magnification, ×25,000).

(include pinocytosis and phagocytosis) (19,20) of various substances including plasma proteins, drugs, immunoglobulins, and metaloproteins (16). Caveolae are also involved in major processes such as signal transduction (21). The endocytosis of molecules is accomplished through nonspecific (via fluid phase endocytosis or adsorptive mechanisms) or specific receptor-mediated pathways. Once ingested, these substances may undergo transcellular transfer between cellular compartments either by fluid phase, adsorptive, or receptor-mediated mechanisms via caveolae (16). In addition, molecules cross the barriers by facilitated diffusion processes via specific carriers (i.e., GLUT-1, A and L-system amino acid transporters) (22) (Fig 2). These essential nutrients and drugs that use facilita-

Table I. Drug Transport Mechanisms at the Blood-Brain Barrier*

Transport mechanisms	Properties involved in cell membrane permeability
Simple passive diffusion for water soluble drugs	Low molecular weight. (Less than 150–200 KDa)
Simple passive diffusion for lipid-soluble drugs	Drug concentration, oil/water partition coefficient, drug protonation, cellular surface area.
Endocytosis (pinocytosis and phagocytosis) and transcytosis	*Via* caveolae through nonspecific pathways (either by fluid phase or by adsorptive mechanisms) or specific receptor-mediated pathways.
Facilitative diffusion *via* specific carriers	Carrier proteins localized at the plasma membrane showing some drug selectivity (drug concentration gradient).
Drug transporters (i.e., organic anion and cation transporters, nucleoside transporters, P-glycoprotein, multidrug resistance proteins)	Carrier proteins localized at the plasma membrane showing drug selectivity and using some form of energy exchange.

* From Refs 14 and 15.

tive mechanisms include glucose, amino acids, adenosine-like compounds, and antimetabolic nucleotides (14). Several families of membrane-associated drug transporters that have been well characterized in peripheral tissues are also expressed in a polarized manner at the BBB. These transporters are involved in the influx and efflux of drugs that are unable to diffuse passively across the cell membrane on their own (i.e., organic cation, organic anion, nucleoside, ATP-binding cassette transporter proteins) (23). The cerebral endothelial cells also express an array of metabolic enzymes such as several cytochrome P450 isozymes, transferases, and phosphatases that are implicated in drug oxidation and conjugation (24).

The BCSF barrier is formed by the choroid plexus (CP), the major interface between two circulating fluids, with the peripheral blood on one side and the CSF on the other side (25,26). The BCSF barrier is located at the outer epithelial surface of the CP whose cells are sealed by tight junctions (26,27). The CP is located in both lateral ventricles, as well as the third and fourth ventricles, and is characterized by leafy, vascular structures composed of fenestrated capillaries and venules. The main function of the CP is to produce and regulate the composition of the CSF by continual secretion and replacement of this fluid. The total volume (140 ml) of CSF is replaced 4 to 5 times daily (28). Similar to the BBB, the CP displays an asymmetric array of receptors, ion channels, enzymes, and transporters with the purpose of maintaining and regulating the homeostatic composition of the CSF (Fig. 3). Facilitative carriers for nonelectrolytes, ion channels for Cl^- and K^+, glutamate transporters, as well as Na^+/K^+ ATPase pumps and Na^+/HCO_3^- cotransport carriers are expressed in a polarized manner along the CP (28–30). Expression of drug transporters such as P-gp, multidrug resistance proteins (MRPs), organic cation, organic anion, and nucleoside transporters have been reported in CP (31–37). In addition, in rat CP, a variety of cytochrome P450 isozymes have been detected along with oxidases, transfer-

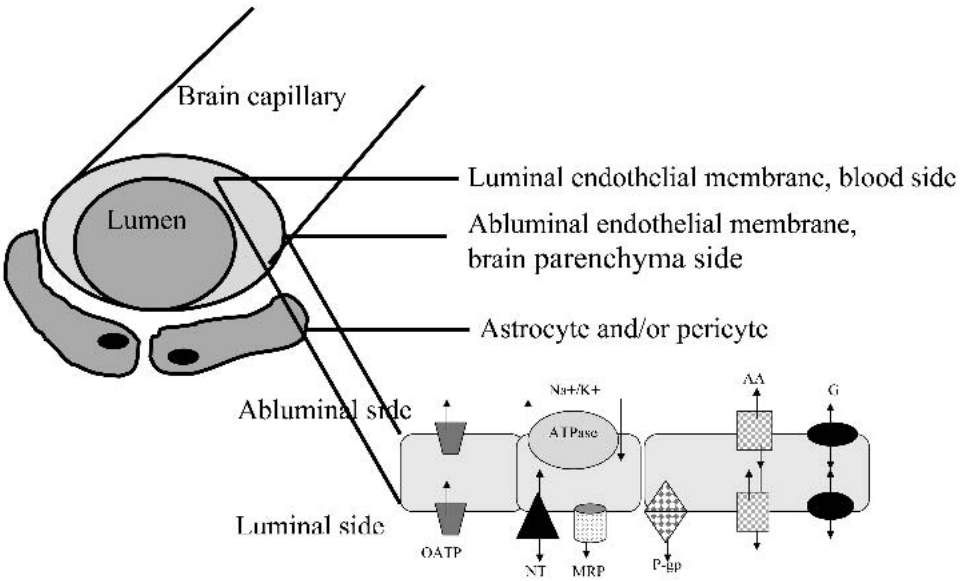

Fig. 2. Transport mechanisms along the blood-brain barrier. A schematic depiction of a cerebral endothelial microvessel showing the luminal and abluminal membranes; the microvessel is surrounded by neighboring astrocyte foot-processes and/or pericytes. A magnified schema further depicts the polarized expression of transporters on cerebral endothelial cells. The arrows indicate the direction of transport. OATP, organic anion transport proteins; NT, nucleoside transporters; Na+/K+ ATPase, sodium and potassium ATPase pump; MRP, multidrug resistance proteins; P-gp, P-glycoprotein; AA, amino acids; G, glucose.

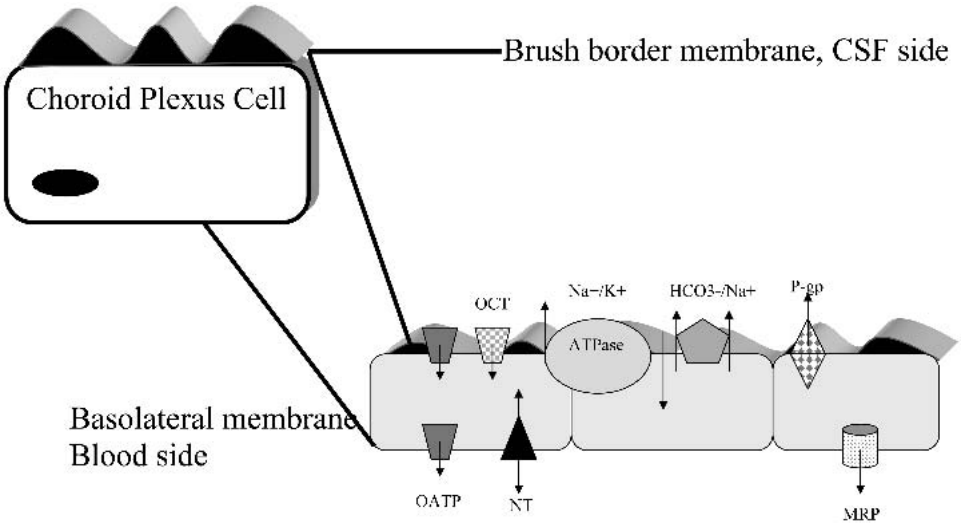

Fig. 3. Transport mechanisms along the blood-cerebrospinal fluid barrier. A schematic depiction of a choroid plexus epithelial cell showing the brush border and basolateral membranes. A magnified schema shows the polarized expression of transporters on choroid plexus epithelial cells. The arrows indicate the direction of transport. OATP, organic anion transport proteins; OCT, organic cation transporter; Na+/K+ ATPase, sodium and potassium ATPase pump; HCO_3^-/Na^+, sodium bicarbonate co-transport carrier; NT, nucleoside transporters; MRP, multidrug resistance proteins; P-gp, P-glycoprotein.

ases, and antioxidants such as glutathione peroxidase (24,38–40). The presence of metabolic enzymes at the BCSF barrier suggests the importance of these structures in cerebral detoxification processes but may also prove to be an obstacle for achieving therapeutic concentrations of active drugs within the CNS.

Brain Parenchyma

Brain parenchyma is composed of neuronal and non-neuronal, or glial, cells. Glial cells are classified into two categories: macroglia and microglia (41). Macroglia are composed of astrocytes and oligodendrocytes and provide a variety of functions to aid in brain homeostasis, whereas microglia cells are derived from the macrophage lineage and provide the primary immune functions of the brain.

Astrocytes represent the principal macroglia cell in the brain. There are two main types of astrocytes: fibrous and protoplasmic. These have been categorized on the basis of morphology, surface staining properties, and brain distribution. Most fibrous astrocytes do not bind tetanus toxin or stain positive for the A2B5 monoclonal antibody (42,43). Fibrous astrocytes possess long, slender, cytoplasmic processes and are expressed in high concentrations around blood vessels and in sub-ependymal areas (44). Protoplasmic astrocytes bind both tetanus toxin and stain positive for the A2B5 antibody. They possess thick, short processes, and are expressed in the cortical gray matter, corpus striatum, and the cerebellum (45). Both types have a relative star-shaped morphology and contain numerous cytoplasmic fibrils, of which the glial acidic fibrillary protein (GFAP) is the main constituent (46). It was originally believed that the primary function of astrocytes was to provide cytoskeletal support for neurons; however, over the years it has been appreciated that these star-shaped cells have important physiological properties in maintaining CNS homeostasis (47). Briefly, astrocytes are involved in regulating neuronal functions as well as guiding neuronal development (48,49). They are involved in the metabolism of neurotransmitters such as glutamate, regulation of extracellular pH and K^+ levels, modulation of immune reactions, and contribute to the structural and functional integrity of the BBB. The close association with the cerebral endothelial cells to neighboring astrocytes through end-foot processes and the secretion of soluble factors aid in the maintenance of BBB integrity (49–51). These multifunctions are carried out by astrocytes through the release of various cytokines (during an immune response), presence of numerous neurotransmitter receptors (for signaling), secretion of soluble factors (to induce BBB integrity and maturation) and the expression of adhesion molecules (for neuronal development) (52–55). Studies also demonstrate that astrocytes express a variety of transporters for nutrients and pharmacological agents. These include the glucose transporter 1 (56), glutamate transporter 1 (30,57), urea transporter 3 (58), nucleoside transporters (59–61), ryanodine receptors (62), ABCG2 transporter (63), along with the efflux drug transporters, P-gp and MRP (64–67).

Oligodendrocytes are a class of specialized macroglia cells that possess the highest metabolic activity in the brain. Their primary function is to form the insulating myelin sheath that surrounds axons in the CNS; Schwann cells form this myelin sheath in the peripheral nervous system (68). Myelin, an extension of the oligodendrocyte membrane, is a lipid-rich biological membrane that forms multilamellar, spirally wrapped sheaths around neuronal axons to increase the resistance for currents during an action potential (68). In culture, oligodendrocytes are observed as large, flat, membranous sheets that are characterized by numerous cytoplasmic extensions containing microtubules and microfilaments (69). This cytoskeleton, in particular the microtubules, have a variety of important roles that include the extension and stabilization of myelin processes, intracellular sorting processes, and the translocation of myelin basic protein mRNAs to the developing myelin sheath (70–72). The expression of trans-

porters, enzymes, ion channels, and receptors in oligodendrocytes seem to vary according to the differential state of the oligodendrocyte as well as its location in the brain. Proteins are present for the transport of endogenous lipids (73), glutamate (30,57), glucose (74), and cationic amino acids (75). Other proteins expressed by oligodendrocytes are ion channels (76), MRPs (77), and enzymes such as Na^+/K^+-ATPase (78), protein tyrosine phosphatase (79), and glutamine synthase (57). In addition, receptors such as the G-protein coupled, extracellular calcium sensing receptor and the ryanodine receptor are expressed in oligodendrocytes (62,79).

The other class of non-neuronal (or glial) cells is the microglia. Microglia are distributed ubiquitously throughout the CNS and comprise up to 20% of the total glial population in the brain (81). Originally identified and characterized by del Rio Hortega (82), these cells are closely related in function and origin to peripheral macrophages and have been referred to as the primary immune cells of the CNS. In the adult brain, microglia are characterized by multiple functional and morphological states depending on the brain environment (83). The cellular form of microglia that is present in the normal adult brain is the ramified microglia. Morphologically, ramified microglia are characterized by a small (5–10 μm) cell body, with numerous branching processes that radiate from the body. Resting microglia constitutively express the complement type-3 receptor (CR-3) and galactose-containing glycoconjugates that bind isolectin B4 (84). Though ramified microglia were generally considered as inactive macrophages, it has been suggested that they may contribute to the normal operation of the brain by participating in extracellular fluid cleansing and transmitter inactivation (85–87). Upon injury or infection, ramified microglia are capable of converting into an activated state. Activated microglia are distinguished by a larger cell body with shorter processes and exhibit partial macrophage-like characteristics such as cell surface markers that include CR-3 and class I major histocompatibility complex antigen (88–90). During an immune response, activated microglia are further transformed to a reactive state. These microglia are typically small and spheroid in shape, but can also exhibit a rod-shaped morphology. In addition to the expression of CR-3 and class I major histocompatibility complex, reactive microglia express class II major histocompatibility complex and several integrins ($\alpha_5\beta_1$, $\alpha_6\beta_1$, $\alpha_M\beta_2$). The defining characteristic of fully reactive microglia is the absence of ramified-type processes and the presence of phagocytic activity (91–93). In addition to the phagocytosis of debris and foreign material during an immune response, reactive microglia secrete a variety of proinflammatory mediators such as cytokines (tumor necrosis factor-α, interleukin-1), reactive oxygen and nitrogen intermediates, and arachidonic acid metabolites, to name a few (94–96). It is believed that the overproliferation and excessive secretion of these potentially neurotoxic factors by reactive microglia may result in the death and dysfunction of surrounding neurons that is a common feature in a variety of pathological disease states such as Wernicke-Korsakoff syndrome, Parkinson's disease, Alzheimer's disease, and HIV-1 associated dementia (91,97–99). Depending on the functional state, microglia express a wide variety of ion channels (K^+, Ca^{2+}, Na^+) (100,101), glutamate receptors (102), glucose transporter 1 (74), nucleoside and organic cation-like transporters (103,104), and the efflux drug transporters P-gp and MRPs (77,105–107).

FUNCTIONAL EXPRESSION AND LOCALIZATION OF P-GLYCOPROTEIN IN THE CENTRAL NERVOUS SYSTEM

P-glycoprotein

P-gp, a member of the ATP-binding cassette superfamily of transporters, is a 170-kDa efflux transporter encoded by the MDR gene (108,109). MDR is a phenomenon where cell lines exhibiting this phenotype have been selected for resistance to a single cytotoxic agent but display a broad cross-resistance to a large number of unrelated drugs, particularly in cancer, HIV, and epilepsy (110). MDR1 and MDR2 genes encode for the P-gp protein in humans (111). In rodents, P-gp is encoded by the *mdr1a, mdr1b, and mdr2* genes (112,113). The human MDR1 and rodent mdr1a and mdr1b gene products confer the MDR phenotype whereas the MDR2 and mdr2 P-gps are primarily involved in phosphatidyl choline excretion into bile across the liver (114–116). The human MDR1 gene product is a 1280-amino-acid-long plasma membrane-associated glycoprotein consisting of two homologous halves (117). Each half contains six hydrophobic transmembrane domains with an ATP-binding site. Primary sequences also reveal extracellular glycosylation sites as well as intracellular phosphorylation sites (118,119).

Although a physiological role for P-gp has yet to be determined, it is believed that the main function of this efflux transporter is to protect the body against potentially harmful xenobiotics from accumulating in the cells. Numerous studies have demonstrated the expression of P-gp in normal tissues such as the intestine, kidney, BBB, BCSF barrier, blood-testis barrier, pancreas, and the peripheral immune cells of various species, including humans. In the periphery, human MDR1 P-gp is localized in a polarized manner to the apical surface of epithelial cells of the intestine, biliary canalicular membrane of hepatocytes, and kidney epithelial cells (120). P-gp is also expressed in the adrenal gland, placental trophoblasts, as well as the secretory and gestational endometrium of humans (120,121). P-gp is expressed in peripheral immune cells such as $CD34^+$ hematopoietic progenitor cells, $CD56^+$ natural killer cells, and in $CD8^+$ cytotoxic T cells (122–124).

It is believed that P-gp confers drug resistance by active, ATP-dependent, extrusion of xenobiotics from the cell. How the transporter actually achieves this process remains speculative. One hypothesis suggests that P-gp, like the classical model for a transporter, functions as a transmembrane pore-forming protein within the lipid bilayer and interacts with certain molecules in the cytoplasm of the cell and expels them directly into the extracellular medium. According to the "hydrophobic vacuum cleaner" model, P-gp binds directly with substrates in the plasma membrane and pumps them out (125). In the "flippase" model, P-gp interacts with molecules in the inner leaflet of the plasma membrane and flips them to the outer leaflet from which they diffuse into the extracellular medium (126,127). This model is consistent with the mdr2 protein, which acts as a flippase for phosphatidylcholine (128). Whether P-gp transports substrates by a combination of several different methods remains to be elucidated.

P-gp substrates are so diverse that it is difficult to identify any structural features that they may share. These substrates include a wide variety of chemotherapeutic agents of natural origin such as anthracyclines (doxorubicin), vinca alkaloids

(vinblastine, vincristine), epipodophyllotoxins (etopside), and taxanes (paclitaxel) (129). P-gp substrates also include drugs and pesticides such as the immunosuppressive agents cyclosporin A and FK506 (130), cardiac glycosides such as digoxin (131), antibiotics like rifampicin (132), HIV protease inhibitors (133), and the antihelmintic pesticide ivermectin (134). Other exogenous P-gp substrates include technetium (^{99m}Tc) sestamibi and rhodamine 123, both of which have been used in imaging and in surrogate marker assays of P-gp function in cells (135,136).

Compounds that inhibit P-gp function include quinolines (quinine), immunosuppressive agents (cyclosporin A), antibiotics (cefoperazone and rifampicin), steroids and hormonal analogs, vinca alkaloid analogs, calcium channel blockers, and calmodulin antagonists, among others (137,138). Most of these agents were identified in the 1980s and have often led to disappointing clinical results due to their low binding affinities, low specificity for P-gp, and high toxicities (139). Second-generation P-gp inhibitors include dexverapamil (less cardiotoxic structural analog of verapamil), dexniguldipine (B859-35; a dihydropyridine derivative), valspodar (PSC833; nonimmunosuppressive analog of cyclosporin A), and biricodar (VX-710; a free-base, nonmacrocyclic ligand). These agents were designed to be less toxic and more potent than the first-generation compounds (3,140). They also have their limitations and affect cytochrome P450 enzyme activity and are not exclusively specific for P-gp (1,2). Third-generation inhibitors include the anthranilamide derivative tariquidar (XR9576), cyclopropyldibenzosuberane zosuquidar (LY336979), the substituted diarylimidazole OC144-093, and acridonecarboxamide GF 120918 (4–7,141,142). So far, these agents have shown to have little or no effect on metabolic enzymes at relevant concentrations and typically have greater specificity for P-gp than their counterparts. Their safety and efficacy will need to be further determined in clinical trials.

Functional Expression and Localization of P-glycoprotein at the Blood-Brain Barrier

P-gp is expressed and functionally active at the BBB, on both the luminal endothelial cells as well as on astrocyte-foot processes on the abluminal side of brain capillaries. Because the surface area of the BBB is about 5000-fold greater than that of the BCSF barrier, the BBB is regarded as the primary route of transport for endogenous and exogenous ligands into the brain parenchyma. The functional expression of P-gp at this barrier poses major implications for the CNS accumulation and distribution of pharmacological agents used in the treatment of neurological diseases.

At the BBB, the functional activity and protein and gene expression of P-gp have been investigated in many species including human, rat, porcine, bovine, and mouse (143–154). The expression of P-gp has been demonstrated in numerous studies at the level of the BBB in freshly isolated brain microvessels, primary endothelial cultures, and in immortalized endothelial cell lines. Studies employing immunohistochemistry and luminal membrane isolation have localized P-gp to the luminal surface of the brain endothelium (143,146,155–158). More specifically, our laboratory has investigated the cellular and subcelluar localization of P-gp in a well-characterized immortalized rat brain endothelial cell line (RBE4) and *in situ* in rat brain cortex by immunocytochemistry using immuno-gold labeling and electron microscopy (154). The results show that P-gp is expressed along the plasma membrane, nuclear envelope, and plasmalemmal vesicles of rat brain endothelial cells. Studies also demonstrate that the level of P-gp expression varies between freshly isolated tissue, primary cell cultures, and continuous cultures of endothelial cells. Regina *et al.* (151) showed that P-gp expression was greatest in freshly isolated brain capillaries, whereas the immortalized rat brain endothelial cell line, GPNT, exhibited higher P-gp expression than the primary rat brain endothelial cells or the immortalized RBE4 cell line. Furthermore, expression of P-gp isoforms also differs depending on the brain tissue. *mdr1a* P-gp encoding mRNA was specifically detected in brain microvessels whereby the *mdr1b* mRNA was found primarily in brain parenchyma (152,155,159). Results from RT-PCR also indicate that in rat brain endothelia, the P-gp isoform *mdr1a* is down-regulated in culture whereas the expression of *mdr1b* is up-regulated (152).

In addition to the luminal localization of P-gp at the BBB, P-gp has been identified at the abluminal side on neighboring astrocyte foot-processes (160). In this study, brain tissues and brain capillaries were labeled with antibodies directed against astrocytic GFAP, MDR1-type P-gp, and the brain endothelial marker GLU1. P-gp was co-localized with the GFAP protein on the abluminal face of the human brain microvasculature rather than being co-localized with GLU1 on the endothelial luminal membrane suggesting that P-gp may be localized at astrocyte foot processes on the abluminal side of the brain microvasculature (160). Although these results are in contradiction to the many studies that have detected *MDR1* in endothelial cells of both normal and tumor brain capillaries (156,161,162), functional studies and Western blot analysis in cultures of primary rat astrocytes do in fact show that P-gp protein is expressed and functional in astrocytes (65–66). Debate remains over whether P-gp is expressed at both sides of the BBB or if the discrepancies arise from varying capillary isolation methods.

In drug-resistant culture cells as well as brain microvessel endothelial cells, P-gp is expressed along the plasma membrane in specialized microdomains termed caveolae (163–165). More specifically, caveolae are present along the plasma membrane of brain capillary endothelial cells and serve to transport macromolecules between cellular compartments by transcytosis (166). By isolating caveolae from brain capillaries through subcellular fractionation using a sucrose gradient, Demeule *et al.* (165) found that P-gp was co-localized with caveolin-1, one of three structural proteins for caveolae for which genes have been identified (caveolin 1, -2, and –3). The exact functional relationship between P-gp and caveolin-1 at the BBB remains to be established.

Recently another member of the ABC superfamily of transporters, the human ABCG2 (also termed the breast cancer resistance protein, BCRP) originally discovered in breast cancer cells (167), was found to be highly expressed at the luminal surface of the human BBB (63,168). A porcine homolog of ABCG2, named brain multidrug resistance protein (BMRP), showing 86% amino acid identity to ABCG2, has also been discovered (169). This protein appears to have the highest expression at the BBB compared to other cell types in the brain (170). Both proteins posess half of the MDR1 P-gp structure with only six predicted transmembrane domains and one ATP-binding domain (167). In addition to the structure

similarity shared by these transporters with MDR1 P-gp, most known ABCG2/BMRP substrates are similar to those of P-gp substrates (171). The functional expression of these transporters at the BBB suggests a possible cooperative role in modulating drug delivery to the brain with P-gp.

Functional Expression and Localization of P-glycoprotein at the Blood-Cerebrospinal Fluid Barrier

P-gp has been localized to the subapical side of the CP epithelia (37). Though P-gp is believed to behave as an excretory pump in tissues such as the kidney, liver, and the BBB to protect the organs against potentially toxic xenobiotics, the apical localization of P-gp at the CP suggest that P-gp may serve to prevent the export of certain substances out of the CSF, opposing the action of P-gp at the BBB in the elimination of xenobiotics from the CNS.

Functional Expression and Localization of P-glycoprotein in Brain Parenchyma (Microglia, Astrocytes, Oligodendrocytes, Neurons)

In addition to the BBB, the functional expression of P-gp has also been detected in some cells of the brain parenchyma such as astrocytes (65–66,172) and microglia (105,172). Decleves *et al.* (65) noted that in primary cultures of rat astrocytes, P-gp protein is expressed but at lower levels when compared to primary rat brain endothelial cultures. This weak expression in astrocytes correlated with a weak functional activity. These results suggest that even if P-gp is expressed at the BBB on astrocyte foot-processes, the higher expression of P-gp at the endothelial level may be more important at limiting the entry of xenobiotics into the brain than at the astrocyte level. In a study by Speigl-Kreinecker *et al.* (173), where significant levels of P-gp were undetectable in healthy human astrocytes (n = 1) cultured *in vitro*. It is worth noting that although these cultures did not express detectable levels of P-gp, high levels of MRP1 were observed. In addition, the ABCG2 transporter is expressed in human fetal astrocytes, although the expression was 3- to 4-fold lower than that of MDR1 P-gp expression (63).

In primary cultures of rat brain microglia, both *mdr1a* and *mdr1b* genes are expressed (106,172), whereas only the *mdr1b* gene is expressed in a continuous rat brain microglia cell line, MLS-9 (105). Using immunogold cytochemistry at the electron microscope, our laboratory has also shown that P-gp is localized to the plasma membrane and nuclear envelope of MLS-9 cells. Western blot analysis show that a band in the range 170–180 kDa is detected in MLS-9 cells, a molecular weight previously reported for P-gp in other cells (152,174). Results from transport studies employing the known P-gp substrate, digoxin, in the presence of various P-gp inhibitors suggest that P-gp is functionally active in the rat brain microglia cell line (105). Furthermore, our laboratory has recently demonstrated the cellular/subcellular location, molecular expression, and activity of P-gp in primary cultures of rat astrocytes (66). These results raise the possibility that the low CNS concentrations of a number of xenobiotics may result from P-gp activity in the brain parenchyma in addition to the BBB.

To the best of our knowledge, there are no reports describing the functional expression of P-gp in healthy oligodendrocytes or neurons to date.

P-GLYCOPROTEIN AND NEUROLOGICAL DISORDERS

In vitro and *in vivo* studies in mdr1a(−/−) knockout mice demonstrate that a broad spectrum of drugs, from antineoplastics to anti-HIV drugs, are P-gp substrates (134,175). This broad substrate specificity may pose significant problems in the clinical setting for a number of common neurological disorders as will be discussed below. In many diseases where unsatisfactory results from drug therapy are observed, investigating the expression of P-gp in these cells is of primary importance. In common brain pathologies such as brain neoplasia, HIV-associated dementia, and epilepsy, P-gp is overexpressed in specific areas of the brain. In most of these cases it is still uncertain whether the up-regulation of P-gp in target cells is due to the presence of the disease itself or occurs during drug therapy, resulting in cellular resistance to the pharmacological agents. Nonetheless, the overexpression of P-gp in brain tissues of cancers, HIV-infection, and epilepsy appears to play a significant role in the altered accumulation and distribution of pharmacological agents used in the treatment of these diseases. In neurological disorders such as Alzheimer's and Parkinson's, the expression levels of P-gp in the brain have been implicated in the etiology and/or progression of the disease.

Brain Neoplasia

MDR is a phenomenon where cells become resistant to a broad spectrum of unrelated drugs with differing chemical structures (110). Tumor cells, including brain neoplasias, are known to develop MDR quite rapidly, leading to serious obstacles in the successful pharmacological treatment of these cancers. Unfortunately for many brain cancers, the success rate of chemotherapy has been very modest, and the prognosis for the majority of these patients continues to be poor (176). A large proportion of primary brain tumors initially respond to chemotherapy, but in most cases these initial responses are short-lived and the efficacy of chemotherapeutic intervention is minimal, particularly for patients exhibiting neuroepithelial tumors that are malignant in approximately 40% of cases (neuroepithelial tumors constitute about half or greater of primary brain tumor cases) (176). There are two main forms of MDR found in tumor cells: intrinsic and acquired. Intrinsic, or *de novo*, resistance is present before exposure to any chemotherapeutic agents and therefore results in initial treatment failure. Resistance developed during the course of chemotherapy is referred to as acquired, and these patients commonly experience early disease progression despite an initial treatment response (176). Unfortunately, many of the drugs associated with MDR are currently used in the treatment of human brain cancers.

Many different mechanisms leading to chemotherapeutic resistance in brain tumors have been postulated and investigated by various groups, with P-gp overexpression being a major component of study and interest. The role of P-gp in brain tumor resistance is compelling because P-gp is present at the BBB, astrocytes, and microglia to prevent the accumulation of potentially toxic substances in the CNS. Cancer of these brain compartments (astrocytes, oligodendrocytes, ependydma, choroid plexus) also constitutes about 50–60% of all primary human brain tumors (176) (Table II). Further-

Table II. Primary Human Brain Tumors

Histologic brain tumor types	Frequency %
1. Neuroepithelial tumors (astrocytoma, oligodendroglial tumor, mixed glioma, ependymal tumors, choroid plexus tumor)	50–60
2. Primitive neuroectodermal tumors (medulloblastoma, ependymoblastoma, pinealoblastoma, neuroblastoma)	2–6
3. Meningeal and related tumors (meningioma, anaplastic meningioma)	15–20
4. Acoustic neuroma (schwannoma)	5–8
5. Primary CNS lymphoma	0.5–2
6. Intracranial germ-cell tumors (germinoma, non-germinomatous germ-cell tumor)	0.5–1
7. Brain tumors of disordered embryogenesis (craniopharyngioma)	2.5
8. Pituitary gland tumor (adenoma, carcinoma)	7–9

Adapted from Bredel *et al.* (176).

more, P-gp substrates include a wide variety of currently used chemotherapeutic agents such as anthracyclines (doxorubicin), vinca alkaloids (vinblastine, vincristine), epipodophyllotoxins (etopside), and taxanes (paclitaxel) (129). In fact, epipodophyllotoxins and vincristine have been used in the treatment of brain tumors with very poor clinical success. The precise association of P-gp overexpression and treatment failure still remains unclear and must be further investigated. Although P-gp expression in brain neoplasia will be briefly discussed in this section, a more comprehensive discussion can be found in a review by Bredel (176).

Reports of P-gp gene and protein expression in brain tumor cells have been inconsistent to say the least. Several groups have reported that a considerable proportion of brain tumors express high levels of P-gp (177–179), whereas in other studies MDR1 overexpression has not been a consistent finding (180–182). Interestingly, Bossayni *et al.* (183) reported that the frequency of P-gp expression generally increased with tumor grade in astrocytic neoplasms where they observed about 30% of low-grade astrocytomas stained for P-gp vs. 88% of high-grade lesions stained for P-gp. Yokogami *et al.* (184) on the other hand, observed that P-gp levels seemed to be inversely related to tumor grade. There are also conflicting results concerning the localization of P-gp within brain tumor tissue, whether or not it is primarily expressed along the plasma membrane of tumor cells or along the luminal membrane of the blood-tumor barrier or at both sites. Sawada *et al.* (162) reported that P-gp was expressed in newly formed microvessels of 16 gliomas but not in 4 meningiomas examined. This finding was confirmed by Toth *et al.* (161) who found P-gp to be expressed in both capillary endothelial cells of normal brain tissue and glioblastomas but not in other type of tumors. In contrast, Tanaka *et al.* (185) reported that P–gp was only detected along the tumor microvasculature but not in the tumor cells themselves. Korshunov *et al.* (186) detected P-gp in both plasma membranes and microvasculature of glioblastomas. The detection of P-gp in brain tumor capillaries has raised the concern that these capillaries are "leaky" compared to normal brain capillaries, and the question of whether P-gp is the primary mechanism in drug efflux has been of interest to some groups. Gallo *et al.* (187) studied the distribution of P-gp substrate paclitaxel, a known P-gp substrate, in brains of wild type and mdr1a/1b(–/–) mice bearing an intracerebral B16 melanoma. They reported that paclitaxel steady-state brain concentrations were significantly greater in P-gp knockout mice compared to wild type and that P-gp plays a role in limiting drug penetration in brain tumors. On the other hand, histological and biological studies undertaken by another group in gliomas showed that *MDR1* gene was not expressed in tumor cells or tumor microvasculature but exclusively in normal endothelial cells (184). Decleves *et al.* (188) examined the functional expression of P-gp in cultured cell lines derived from patients with glioblastoma multiforme, a highly malignant cancer of the CNS, often refractory to chemotherapy (189). In these particular cancer cell lines, MDR1 P-gp was not detected by Western blot analysis or by RT-PCR although other efflux transporters such MRP1, 3, 4, and 5 were detected. Demeule *et al.* (155) investigated the presence of MDR1 P-gp in 60 human brain tumors by Western blot analysis. Tumor samples included meningiomas, schwannomas, low-grade gliomas (astrocytomas, pilocytic astrocytomas), and high-grade gliomas (anaplastic astrocytomas, glioblastomas, and anaplastic oligodendrogliomas). This study reports that most primary brain malignant gliomas express P-gp at the same levels as those in normal brain homogenate, whereas secondary brain tumors from lung adenocarcinomas and melanomas had lower P-gp levels than normal brain. The expression of P-gp in malignant brain tumors confirms results from previous studies and suggests that P-gp may be related to the poor chemotherapeutic response of these tumors (178,180,189–191). The highest levels of P-gp were detected in benign meningiomas and may provide a marker for chemotherapy outcomes.

It is important to note that differing experimental methods and conditions for detecting P-gp were used in these studies (RT-PCR, immunohistochemistry, Northern blot, sensitivity of antibodies used, sample size) and may be a factor contributing to these conflicting results. Moreover, interindividual variability must also be taken into account for the different findings observed. These conflicting results suggest that P-gp may not be expressed in every form of brain tumor. In fact, the expression of P-gp seems to differ between primary and metastatic, malignant and benign, as well as between classes and even subtype of the brain tumor itself. Additional studies into specific classes and subsets of brain tumors may be helpful in determining exactly which tumors will or will not respond to various chemotherapeutic regimens depending on their tendency to overexpress P-gp.

HIV-Associated Dementia

Although the incidence of HIV-associated dementia (HAD) has decreased in developed countries with the advent of highly active antiretroviral therapy, commonly known as HAART, complete protection or reversal of this disorder has not yet been achieved (192). Prior to HAART, the collective

risk of developing HAD during an HIV-infected person's lifetime was about 15–20% (192); however, the exact risk of developing HAD in the HAART era is currently unknown (194). An important risk factor recently observed for HAD is age. Data from the Hawaii Aging with HIV cohort reported that HIV-positive individuals who were greater than 50 years were almost twice as likely to meet criteria for HAD as younger HIV-infected individuals (195). It is believed that this prolonged survival for HIV infected patients, along with the increased risk for HAD, may lead to an increased prevalence of HAD within the population (196). HAD is a cognitive and motor disorder that results from HIV-1 infection of the CNS. HAD is characterized by impaired memory, psychomotor slowing, and paraplegia, among other symptoms that eventually lead to disabling cognitive and motor dysfunction (197). The primary target for HIV-1 infection in the brain is microglia, the resident immune cells of the brain, and to a lesser extent, astrocytes (198,199). HIV-1 infection of microglia results in an amplified inflammatory process involving the secretion of various cytokines (interleukins 1 and 6 and tumor necrosis factor-α) and neurotoxins such as glutamate, quinolinic acid, platelet-activating factor, nitric oxide, and arachidonic acid metabolites (200,201). These factors can be toxic to neurons as well as induce astrocytosis (202). This inflammatory reaction propagated by HIV-infected microglia and astrocytes leads to, over time, the neuronal loss or dysfunction that underlies HAD (203).

The addition of protease inhibitors (PIs) to the current HIV drug regimen has significantly reduced plasma viral loads in HIV-positive patients. In spite of these promising results, treatment failure and the emergence of resistant strains of HIV-1 continue to pose a major problem, particularly in the treatment of HAD. PIs exhibit low bioavailability following oral administration and penetrate poorly into the CNS (204,205). One possibility for the low brain permeation of these antiretrovirals may be due to the presence of P-gp and MRPs. *In vivo* and *in vitro* studies have clearly shown that PIs are substrates for P-gp and possibly the MRPs (133,175,206–208). P-gp overexpressing cells, G185 cells, are resistant to saquinavir-mediated cytotoxicity which can be reversed by the administration of P-gp inhibitor cyclosporin A (208). In the same study, it was demonstrated that there is specific, directional transport of saquinavir in an epithelial monolayer cell line, and transport was blocked in the presence of P-gp inhibitors cyclosporin A and verapamil (208). Similarly, Alsenz *et al.* (206) studied the *in vitro* transport of saquinavir and ritonavir in the human Caco-2 cell system expressing Pgp on its apical surface. This study demonstrated that both PIs are transported through the Caco-2 cell monolayers in the basolateral to apical direction and is inhibited by verapamil and cyclosporin A. Kim *et al.* (175) showed that the basal-to-apical transport of the HIV-1 PIs nelfinavir, saquinavir, and indinavir across Caco-2 cells is reduced significantly with the co-administration of P-gp inhibitors quinidine and PSC 833. Our laboratory has also confirmed these results by showing that saquinavir and indinavir are transported by P-gp in both RBE4 and MLS-9 cell lines (67). Perhaps most informative is that *in vivo* brain concentrations of PIs were elevated 4- to 36-fold in mice lacking the *mdr1a* gene (175,209). In mice expressing the *mdr1a* gene, brain concentrations of nelfinavir were increased up to 37-fold by addition of the potent cyclopropyldibenzosuberane P-gp inhibitor, LY-335979 (210). In addition to being substrates for P-gp, PIs also demonstrate P-gp inhibitory effects with ritonavir being one of the most potent. In a study by Lee *et al.* (133), photoaffinity labeling of the P-gp substrate [^{125}I]iodoarylazidoprazosin was inhibited by saquinavir, indinavir, and ritonavir in a concentration-dependent manner. In addition, this group demonstrated that the PIs significantly stimulated P-gp-specific ATPase activity, and this was inhibited by the administration of the P-gp inhibitor PSC 833. Our studies also showed that saquinavir, indinavir, and ritonavir could inhibit the transport of the P-gp substrate digoxin across RBE4 and MLS-9 cell lines (105,154).

Although HIV disease progression has considerably been reduced in patients taking HAART, treatment outcomes are not always satisfactory, and P-gp has been implicated in playing a role in resistance to HIV drug therapy. Whether the HIV virus or the antiretrovirals themselves are responsible for the up-regulation of P-gp in HIV-infected cells is still under investigation. An early study by Gollapudi and Gupta (211) examined whether HIV could induce P-gp expression in HIV infected cells. They reported that P-gp expression was up-regulated upon HIV infection of a T cell and monocytic cell line. The functional activity of P-gp was demonstrated by showing that HIV-infected cells accumulated less 3′-azido-3′-deoxythymidine and daunorubicin as compared to uninfected cells. Andreana *et al.* (212) also reported P-gp up-regulation in peripheral blood mononuclear cells (PBMCs) from HIV-infected patients. Interestingly, they observed a functionally defective P-gp in HIV-infected cells that increased with progression of HIV-infection. Recent studies, however, report the down-regulation of P-gp in HIV-infected cells compared to controls. Malorni *et al.* (213) investigated P-gp expression in PBMCs from HIV-infected patients and reported that P-gp expression was decreased in subsets of PBMCs (T-CD8$^+$ and NK-CD16$^+$) in HIV-infected cells. Meaden *et al.* (214) and Agrati *et al.* (215) reported similar findings. Other studies suggest that antiretrovirals may be responsible for P-gp up-regulation in HIV-infected cells. Various groups have reported that some PIs (saquinavir, ritonavir, nelfinavir, amprenavir) and non-nucleoside reverse transcriptase inhibitors (efavirenz) induce significant P-gp expression in peripheral immune and intestinal cells that have been cultured in these drug solutions for a period of time (216–220). Still other groups have reported that PIs do not enhance P-gp expression and suggest that resistance to PIs, such as saquinavir, is associated with mutations in the HIV protease enzyme rather than an overexpression of P-gp (221,222). Similarly, studies have shown that the induction of drug resistance by selection with increasing concentrations of zidovudine resulted in cells that were resistant to zidovudine but did not express detectable amounts of P-gp (223). It has been proposed that resistance to zidovudine is primarily associated with mutations in HIV reverse transcriptase and not P-gp overexpression (224).

In brief, results from *in vitro* and *in vivo* studies suggest that PIs are P-gp substrates and that P-gp may limit the penetration of these drugs into the CNS resulting in the ineffective and incomplete treatment of HIV infection in the brain. Furthermore, the presence of the HIV-1 virus and/or anti-HIV drugs may be a factor in the up-regulation of P-gp in HIV-infected cells resulting in poor drug accumulation. Therefore, expression of P-gp is probably one of several

mechanisms that contribute to cellular resistance to anti-HIV drugs.

Epilepsy

Epilepsy is a neurological disorder characterized by recurrent seizures that affects approximately 1–2% of the world's population (225). Despite considerable advances in the pharmacological treatment of epilepsy, approximately one-third of epileptic patients are pharmacoresistant (226,227). Although the exact mechanisms are still not fully understood, it has been observed that in some of these pharmacoresistant epileptic patients, blood concentrations of antiepileptic drugs (AEDs) are within normal therapeutic levels. Another important characteristic of pharmacoresistant epilepsy is that these patients demonstrate resistance to several, if not all AEDs, even if these drugs act by differing mechanisms (228). These observations suggest the possibility of decreased drug uptake into the brain by overexpression of multidrug transporters, such as P-gp.

The search for the possible role of efflux transporters in pharmacoresistant epilepsy is a relatively new one. It began with Tishler *et al.* (229), who were the first group to report the overexpression of MDR1 MRNA in the focal brain tissue of patients with pharmacoresistant partial epilepsy. This group further explored P-gp expression in epileptic brains by localizing P-gp to the capillary endothelium in brain tissues of patients with intractable epilepsy. Dombrowski *et al.* (230) also reported the overexpression of P-gp in the capillary endothelium of human epileptic brain specimens. During the years, it has been observed that P-gp and/or MRPs are not only expressed at the BBB but in other areas of epileptic brains. Both P-gp and MRP1 up-regulation has been reported in a range of epileptogenic lesions containing reactive astrocytes (63,229,231,232). In addition, many more studies have reported the up-regulation of either P-gp or MRPs, or both, in neuronal and/or neuro-glia cells in developmental lesions of resected and postmortem human brain tissue of patients with refractory epilepsy (232–235). Rizzi *et al.* (236) showed in rats that P-gp mRNA was up-regulated either in limbic areas following seizures induced by kainate or in rats made spontaneously epileptic by remote status epilepticus. This group in turn observed that repetitive treatment with AEDs did not induce P-gp mRNA expression, as did seizures. This result suggests that the up-regulation of P-gp may not be induced by the presence of AEDs but by some regulatory pathway induced by seizures and that constitutive overexpression of the efflux transporter P-gp may be an important mechanism underlying the intrinsic resistance to epilepsy drugs in lesions of patients with medically intractable epilepsy.

In addition to the overexpression of P-gp in epileptic brain tissue, several animal studies also report that some AEDs may in fact be substrates for P-gp and MRP transport. The AEDs include phenytoin (229,236–238), carbamazepine (236,237), lamotrigine, topiramate, felbamate (237,239), and phenobarbitone (132). Although these studies suggest that AEDs may be P-gp substrates, it is important to take into account some experimental limitations, hence the data should be carefully interpreted (132,229). For example, *in vivo* and *in vitro* studies using certain P-gp inhibitors should take into account the fact that the P-gp inhibitor verapamil is a cerebral vasodilator and not a very specific inhibtor of P-gp. In another study, it was reported that the vehicle used for PSC833 affected P-gp-mediated transport of its substrate (238). Owen *et al.* (240) assessed the role of P-gp in carbamazepine transport in mdr1a/1b(–/–) mice, in Caco-2 cells in the presence of PSC833, and by flow cytometry using the P-gp substrate rhodamine 123. In all three systems, this group reported that carbamazepine was not a substrate for P-gp.

Taken together, the limited data on the expression of P-gp in brain tissues of patients with refractory epilepsy and the questionable role of P-gp in the transport of AEDs across cellular membranes demonstrates this area requires further thorough investigation.

ALZHEIMER'S DISEASE AND PARKINSON'S DISEASE

Alzheimer's Disease

There are over 15 million cases of Alzheimer's disease (AD) worldwide, and AD is the most frequent cause of dementia in the elderly (241). AD is a progressive dementing neurologic disorder that is characterized by significant neuronal cell loss and brain atrophy primarily in the hippocampus and cerebral cortex (241,242). The two main neuropathologic features of AD are the neurofibrillary tangles and neuritic plaques, of which there are two types: neuritic plaques consisting of neuronal processes surrounding a fibrillar β-amyloid core and diffuse plaques. The neuritic plaques are primarily composed of the β-amyloid protein (Aβ) and are believed to be the site of local inflammatory processes due to the presence of acute phase proteins, reactive microglia, cytokines, complement components, and protease enzymes (243,244). The excessive accumulation of these Aβ proteins have been implicated in the pathogenesis of AD (245). Evidence suggests that the accumulation of Aβ initiates a cascade of inflammatory events that include microgliosis and astrocytosis. The activation of microglia and astrocytes results in the secretion and accumulation of neurotoxic factors for which the end result is neuronal dysfunction and death resulting in the clinical manifestations of impaired mnemonic, cognitive, and behavioral functions (242,246). Aβ is approximately 40 or 42 amino acids in length and consisting of 28 hydrophobic amino acids that reside intracellularly and in the interstitial fluid of the brain and 12–14 hydrophobic amino acids that remain associated with the membrane following enzymatic cleavage (247,248). Both the hydrophilic and hydrophobic Aβ peptides are secreted from neuronal and non-neuronal cells, and it is believed that the secretion of the membrane-associated Aβ peptides requires active detachment from the membrane (247,249–251).

Because P-gp has been shown to actively transport a wide range of lipophilic and amphipathic molecules from cells, it is possible that this efflux protein could be responsible for the transport of Aβ from brain cells (252–254). Lam *et al.* (247) investigated this hypothesis and found that P-gp may be involved in the active secretion of the membrane-associated hydrophobic Aβ peptides. The secretion of Aβ was investigated in cells transfected with MDR1 in the presence of potent P-gp reversal agents, the antiprogestin/antiglucocorticosteroid RU 38486 (255), and its 17-beta derivative RU 49953 (256). Both compounds significantly decreased Aβ secretion compared to controls. *In vitro* binding studies showed

that synthetic Aβ peptides interact directly with P-gp in highly purified hamster mdr1 reconstituted into membrane vesicles. Furthermore, transport of Aβ peptides across plasma membranes of P-gp enriched vesicles was measured and found to be both ATP-dependent and P-gp dependent.

Subsequent to this study, Vogelgesang *et al.* (248) hypothesized that if P-gp were one of the transporters involved in Aβ secretion out of brain cells, then Aβ accumulation in the brain would correlate inversely with the degree of P-gp expression at the BBB. Brain tissue samples were taken at routine autopsies from 243 nondemented subjects who died between the ages of 50 and 91 years, and P-gp expression at the BBB and the number of Aβ plaques were assessed immunohistochemically. This group reported a significant inverse correlation between P-gp expression at the BBB and the deposition of Aβ plaques suggesting that high levels of P-gp at the BBB may serve as a protective measure by eliminating excessive levels of Aβ peptides out of the brain. Studies have also shown that 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors, simvastatin and pravastatin, can modulate P-gp activity (257) and increase mdr2 P-gp expression in rat bile and liver (258). A study by Fassbender *et al.* (259) showed that these same inhibitors reduced intra- and extracellular levels of Aβ peptides in primary cultures of hippocampal neurons although a definitive correlation with P-gp has not been made. These observations suggest that up-regulation of P-gp activity at the BBB may be an alternative approach to direct drug therapy in eliminating or at least in controlling the accumulation of brain Aβ peptides.

In the United States, four medications have been approved for the symptomatic treatment of AD (tacrine, donepezil, rivastigmine, galantamine) (260–263). They are cholinesterase inhibitors that provide modest benefit in improving symptoms in about 30–40% of patients with mild AD. Unfortunately, these drugs have varying organ toxicities and side effects that limit their use. In fact, tacrine has been removed from the market due to reported liver toxicities. Researchers have also suggested that these drugs may in fact slow the rate of disease progression; however, this remains to be confirmed in prospective longitudinal clinical trials. New therapies that prevent the formation of plaques (264) and strategies to induce immunologic responses capable of removing existing plaques are being investigated (265,266). To date, we are unaware of studies investigating the possible role of P-gp in the transport of these drugs.

Parkinson's Disease

Parkinson's disease (PD) is a common neurodegenerative disorder with an estimated prevalence ranging from 70 to 170 per 100,000 individuals in North America (267). There are two forms of PD: rare familial forms inherited as autosomal dominant or recessive traits and the sporadic form, where environmental factors play a significant role (268,269). A host of environmental risk factors has been investigated, and accumulated data suggest that exposure to pesticides, rural living, well water, and various other drug contaminants such as 1-methyl-4-phenylpyridinium (MPP^+) are associated with PD (270–272). In fact, a study by Betarbet *et al.* (273) reported that chronic exposure of rats to rotenone, an insecticide commonly used in farms and lakes, produced behavioral and neuropathological symptoms consistent with those of PD in humans.

Due to the association of environmental toxins and PD, an interest has developed in the field of genetic polymorphisms of potential host factors, such as transporters and enzymes that may be involved in the uptake, metabolism, and distribution of these toxins and in turn be plausible candidates as PD risk genes. The study of a number of polymorphisms of enzymes that are involved in the biotransformation of a variety of exogenous substances show a significant association with PD, although their pathophysiologic role remains to be determined (274–276). It is also believed that polymorphisms in the P-gp gene may be another indicator for PD risk, as P-gp is responsible for the efflux of toxic compounds out of the brain environment. In fact, *in vivo* studies in mice lacking the *mdr1* gene show that P-gp plays a crucial role in preventing the accumulation of pesticides and potentially toxic substances in the brain (134,175,277,278). This observation suggests that low P-gp expression at the BBB as well as in other brain compartments may increase an individual's risk for PD because the protective mechanism of extruding potentially toxic substances out of the brain is lacking.

To date, little is known about the possible association of P-gp expression and the pathology of these two neurodegenerative disorders such as Alzheimer's and Parkinson's diseases.

SUMMARY

P-gp, a membrane-associated, energy-dependent drug transporter is a glycoprotein involved in the cellular efflux of potentially toxic xenobiotics including pharmacological agents. The expression of P-gp in healthy cells and its polarization at numerous barrier interfaces suggest this protective role. P-gp was first discovered in tumor cells where it contributes to the MDR phenomenon against a number of unrelated anticancer drugs (279). In addition, P-gp expression is up-regulated in a wide range of disorders, particularly neurological conditions including HIV-associated dementia, pharmacoresistant epilepsy, and a variety of brain cancers. Overall, it is believed that P-gp up-regulation is correlated with poor clinical outcomes in drug therapy. The specific mechanism by which P-gp overexpression is regulated in neurological disorders remains unclear and continues to be investigated. In HIV infection, debate exists whether P-gp up-regulation is induced by the HIV-1 virus or by prolonged exposure to antiviral drugs. In pharmacoresistant epilepsy, further research is required to determine if P-gp expression is regulated by seizure-associated mechanisms or by exposure to AEDs. Currently used anticancer drugs are known P-gp substrates. This may account for the modest to poor success rate of chemotherapy in neuroepithelial tumors (176). Because P-gp is not overexpressed in all brain tumors, additional studies into specific classes and subsets of brain tumors are required to establish which tumors will or will not respond to various chemotherapeutic regimens depending on their tendency to overexpress P-gp.

In addition to conferring the MDR phenotype, P-gp has also been found to play an important role in the etiology of disease or in disease progression itself. Recent research in AD suggests that individuals with naturally low levels of P-gp expression in certain areas of the brain may be predisposed to the excessive accumulation of Aβ, the main neuropathologic feature of AD. Because *in vitro* studies have suggested that Aβ may be a substrate for P-gp, methods of up-regulating

P-gp expression at the BBB may serve as a strategy in the prevention and/or treatment of AD. In PD where excessive accumulation of environmental toxins (known to be P-gp substrates) has been implicated in the pathogenesis of the disease, polymorphisms in the P-gp gene may be an indicator for PD risk. Similar to AD, the up-regulation of P-gp in the brain, particularly along the BBB, may serve as a therapeutic approach in the prevention and/or treatment of PD. In brief, the various functions of P-gp highlight the complexities of the overall role of this ABC transporter in the etiology, pathogenesis, and pharmacotherapy of neurological disorders.

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