The Role of P-glycoprotein in the Blood-Brain Barrier

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ABSTRACT

The blood-brain barrier is composed of specialized endothelial cells that prevent various substances from entering the brain. P-glycoprotein is an important component of this barrier and is present in high concentration on the apical surface of these endothelial cells. It is an ATP-dependent transport protein thought to be involved in extruding a variety of structurally unrelated compounds and preventing their accumulation within the brain. It is known that P-glycoprotein impedes the entry of various drugs that are used in the treatment of central nervous system diseases. Understanding the structure and the function of P-glycoprotein will lead to the development of specific and selective P-glycoprotein inhibitors. Combined use of these inhibitors along with therapeutic agents could treat central nervous system diseases and result in improved clinical efficacy.

INTRODUCTION

The existence of a blood brain barrier (BBB) has been known since the 1880s when Paul Ehrlich demonstrated its presence through the use of vascular dyes. Subsequently, in the 1960s, Drs. Reese, Karnovsky, and Brightman localized tight junctions in the endothelial cells of the brain using electron microscopy (Engelhardt, 1997). It is now known that the BBB is composed of specialized endothelial cells that line the capillaries of the brain. The features of these endothelial cells that restrict transport across the BBB are tight junctions between the endothelial cells, the presence of few pinocytotic vesicles, and the lack of fenestrae (reviewed in Doze et al., 2000), (Figure 1, from van Asperen et al., 1997).

The BBB is known to exclude nearly all molecules from entering the brain except those that are either small or lipophilic (reviewed in Rubin and Staddon, 1999). This is essential to protect the brain from exposure to toxic substances and to maintain the proper internal environment for brain function. However, there are specific transporters on the endothelial cells of the brain for small and large hydrophilic molecules that enter the brain by active transport as well as specific membrane transporter proteins for essential nutrients. Membrane proteins also exist on brain capillaries for transporting a number of lipophilic molecules that enter the endothelial cells back to the blood. P-glycoprotein (P-gp) is one such transporter present in high concentration on brain capillaries (Thiebaut et al., 1989; Cordon-Cardo et al., 1989) (Figure 1, from van Asperen et al., 1997) and is an important component of the BBB (Rubin and Staddon, 1999). It is also present on the bile canalicular membrane of hepatocytes as well as kidney, testes, and cells in the intestines (Thiebaut et al., 1987; Thiebaut et al., 1989). It is also known to confer multidrug resistance to tumor cells (Juliano and Ling, 1976; reviewed in Johnstone et al., 2000).

While P-gp is involved in protecting the brain from exposure to a variety of pharmacologically active hydrophobic agents, it is an impediment to the treatment of various central nervous system (CNS) diseases such as primary brain tumors and cerebral human immunodeficiency virus (HIV) infection. For instance, availability of many protease inhibitors for the management of HIV infection has reduced the plasma viral load. However, in tissues such as the brain viral persistence, and the clinical findings associated with acquired immune deficiency syndrome (AIDS) dementia complex continue even in the face of undetectable plasma viral load (Kim et al., 1998). This suggests that perhaps, the viral persistence is due to poor penetration of the BBB by these drugs. Kim et al. (1998) have demonstrated that the HIV protease inhibitors nelfinavir, indinavir, and saquinavir are P-gp substrates whose entry into the brain is limited in wild type mice when compared to mdr1a knockout mice (1998). This supports the idea the P-gp might be involved in preventing the entry of the very drugs that are intended to treat HIV infection of the brain. Therefore, understanding the molecular and the functional aspects of the P-gp will aid in the development of therapeutic agents that can gain entry into the CNS in spite of the actions of this transporter. It would also further the development of those drugs intended for the treatment of peripheral diseases such as tumors without increasing the incidence of neurotoxicity.

P-GLYCOPROTEIN STRUCTURE

Human P-gp is a 170 kDa transmembrane protein encoded by the multidrug resistance gene (MDR1), containing 27 exons spread over 100 kb located on the long arm of chromosome 7 (reviewed in Fardel et al., 1996). It is located on the apical surface of the endothelial cells in brain capillaries. It is also involved in actively extruding various structurally unrelated amphipathic compounds with a molecular weight greater than 500 Da from the endothelial cell into the capillary lumen and preventing their accumulation in the brain. ATP
hydrolysis is required for extruding the drug against its concentration gradient (reviewed in Schinkel, 1999). Human P-gp is composed of 1280 amino acids. The N- and C-terminal 610 amino acids are each organized into 6 hydrophobic transmembrane domains (TMD) followed by a hydrophilic nucleotide-binding domain. These 2 halves are joined by a 60 amino acid linker region (Figure 2). This organization of the domains is characteristic of ATP-binding cassette transporters. P-gp is a member of this family (reviewed in Loo and Clarke, 1999). The 12 TMDs are thought to fold in a barrel-like conformation (Schinkel, 1999).

P-gp is N-glycosylated on the first extracellular loop in three different locations. While these sites are not thought to be involved in substrate specificity or the function of P-gp, the effectiveness of the protein seems to be affected. This is thought to be due to altered protein stability or plasma membrane targeting (reviewed in Borst and Schinkel, 1997). P-gp has many serine or threonine phosphorylation sites that have been shown to be phosphorylated by several kinases including protein kinase C (PKC) and cAMP-dependent protein kinase (reviewed in Fardel et al., 1996). The functional significance of this is not well understood. Phosphorylation of P-gp results in modification of the drug resistance conferred to some cell lines (reviewed in Fardel et al., 1996). However, systematic replacement of serine or threonine with alanine or asparagine does not alter the activity of P-gp (reviewed in Borst and Schinkel, 1997).

P-gp has affinity for a broad range of structurally unrelated large hydrophobic compounds including vinca alkaloids, epipodophytoxins, taxanes, anthracyclines (reviewed in Loo and Clarke, 1999), cyclosporin A, digoxin (reviewed in Schinkel, 1999), and various HIV protease inhibitors (Kim et al., 1998). It is not clear how a single transporter can have such a diverse array of substrates that vary in size and chemical properties. The only unifying structural feature shared among the P-gp substrates is that they contain spatially separated hydrophobic and hydrophilic moieties (reviewed in Schinkel, 1999). Structural details are beginning to emerge that might eventually unravel the mechanism of P-gp export.

Photoaffinity labeling studies suggest that the drug recognition, binding, and translocation of various substrates is mediated primarily by TMD 6 and TMD 12 (reviewed in Loo and Clarke, 1999). Systematic mutation of phenylalanine (F) residues to alanine (A) residues in the putative TMD segments lead to the identification of two mutants with altered drug resistance profile: F335A in TMD6 and F978A in TMD12. These two residues are located in identical positions when the two similar halves are aligned (reviewed in Loo and Clarke, 1999). It has been suggested that TMD 6 and TMD 12 form part of the drug-binding pocket and assist in coupling drug binding to ATPase activity. Cross-linking studies (reviewed in Loo and Clarke, 1999) have shown that TMD 6 and TMD 12 are close to each other and undergo conformational changes during the reaction cycle (Figure 3). The hydrophilic nucleotide-binding consensus sequences bind ATP, and P-gp shows drug-stimulatable ATPase activity. ATP hydrolysis is required for drug transport, and both ATP binding sites are essential for transport. Similarly, experiments have shown that both halves

**FIGURE 1** | A schematic representation of the components of the BBB. The presence of tight junctions between the brain endothelial cells, few pinocytotic vesicles, and the lack of fenestrae are some of the essential features of BBB. Specific transporters exist for shuttling nutrients such as glucose and amino acids. P-gp is localized to the apical membrane of the endothelial cell and actively extrudes a variety of compounds out of the brain and into the capillary lumen (van Asperen et al., 1997).
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of P-gp are required for forming the drug-binding pocket and the presence of drug substrates aid in the proper folding of the two halves (reviewed in Loo and Clarke, 1999). Studies have also shown that drug binding requires N- and C-terminal TMD for the formation of the drug-binding pocket and is independent of the nucleotide-binding domains (reviewed in Loo and Clarke, 1999).

MECHANISM OF P-GLYCOPROTEIN ACTION

How P-gp mediates the transport of various drugs is still unknown. Different models have been proposed to explain the mechanism of P-gp mediated drug transport. The classical model invokes a pore-forming arrangement of the TMDs to suggest that P-gp acts as a transport protein by expelling drugs from the cytoplasm to the extracellular location (reviewed in Borst and Schinkel, 1997). The hydrophobic vacuum cleaner model suggests that P-gp binds directly to the substrates on the plasma membrane and pumps them out of the cell by recognizing them as foreign to the membrane (reviewed in Fardel et al., 1996). Another model proposes a secondary role for P-gp in drug transport: it alters the intracellular pH or the membrane potential by functioning as a proton or chloride pump, and thus secondarily reduces the accumulation of weakly basic cationic lipophilic drugs (reviewed in Fardel, et al., 1996). The flipase model (reviewed in Higgins and Gottesman, 1992) proposes that P-gp encounters the drugs in the inner leaflet of the lipid bilayer and flips them to the outer leaflet against a concentration gradient. From the outer leaflet the drugs then diffuse to the extracellular site (Figure 4, Johnstone et al., 2000). Accumulating evidence points to this last model as the currently favored mechanism of P-gp action (reviewed in Higgins and Gottesman, 1992).

The flipase model is based on the idea that the substrate gains access to the core of the TMDs from the lipid bilayer after its interaction with the membrane, and P-gp flips the drug from the inner to the outer leaflet in an ATP hydrolysis dependent fashion (reviewed in Higgins and Gottesman, 1992). The underlying assumption is that the substrates would intercalate between the phospholipid bilayers prior to the interaction with P-gp. Given that the P-gp substrates are primarily cationic, lipophilic, planar molecules, such an interaction is expected if the molecule is to enter the cell (reviewed in Higgins and Gottesman, 1992). This model explains the existence of a broad spectrum of substrates handled by P-gp because of a two-tier recognition system. The substrate must first intercalate between the

FIGURE 2 | Human P-gp is a transmembrane glycoprotein composed of 1280 amino acids. It is thought to be organized into 2 hydrophobic domains each consisting of 6 transmembrane segments and 2 cytoplasmic nucleotide-binding domains. It has three glycosylation sites on the first extracellular loop (Loo and Clarke, 1999).
lipid bilayer appropriately, and then interact with the substrate-binding region of P-gp. This would allow P-gp to be relatively non-specific in its selection of substrates, since the primary criterion for P-gp binding would be the ability of the molecule to intercalate between the lipid bilayers (reviewed in Higgins and Gottesman, 1992). This model also explains the differential handling of its substrates based on their lipophilicity: a drug with a higher lipid partition coefficient will be more easily removed from the lipid bilayer by P-gp than one with a lower lipid solubility independent of their relative concentrations in the system (reviewed in Higgins and Gottesman, 1992). By intercepting the substrates before their entry into the cytoplasm of the cell P-gp prevents their interaction with more specific intracellular drug targets. Based on the flippase model it has been suggested that the physiological role for P-gp is to remove agents that intercalate and introduce membrane discontinuities (reviewed in Higgins and Gottesman, 1992).

The emerging model for the P-gp mediated drug efflux requires a coordinated action between the two TMDs and the two nucleotide-binding domains. After the substrate enters the lipid bilayer it interacts with the drug-binding pocket formed by residues located on TMDs 6 and 12. Following ATP hydrolysis at alternating sites, a conformational change takes place in the TMDs such that drug affinity is reduced in TMDs 6 and 12 resulting in drug efflux (Figure 3; reviewed in Loo and Clarke, 1999).

FIGURE 3 Working model of P-gp action. Coordinated activity between the two hydrophobic domains and the nucleotide-binding domains is required for P-gp mediated drug efflux. The drug-binding pocket is located in the interface between the two halves of the molecule and is closely associated with TMDs 6 and 12. After a drug enters the lipid bilayer it interacts with specific residues in the drug-binding pocket. ATP hydrolysis follows and a conformational change in the TMDs is coupled to drug efflux (Loo and Clarke, 1999).

CLINICAL IMPLICATIONS

Regulation of P-glycoprotein

P-gp expression has been shown to be modulated under various circumstances. These include upregulation when exposed to xenobiotics and hormones in vivo as well as modulation when exposed to environmental stress, differentiating agents, xenobiotics, and hormones in vitro (reviewed in Fardel et al., 1996). There is a strong correlation between relapse of soft tissue sarcoma and the outcome of neuroblastoma with an increase in the levels of MDR1 gene expression (reviewed in Fardel et al., 1996). Acute treatment of human cell lines with anti-cancer drugs in vitro results in P-gp induction. This is thought to occur because MDR1 promoter responds directly to cytotoxic agents (reviewed in Fardel et al., 1996). Other agents that promote P-gp induction are verapamil, PKC agonists, osmotic shock, low pH, retinoic acid, arsenite, cadmium chloride, steroid hormones, and heat shock (reviewed in Fardel et al., 1996). The modulation of P-gp levels appears to be cell and tissue-specific as demonstrated by the downregulation of P-gp in leukemia cells by verapamil (reviewed in Fardel et al., 1996).

Reversal Agents

This presents a challenge in treating various diseases that affect the CNS. In addition to being extruded by the P-gp already present on the BBB, there is an upregu-
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The therapeutic application of P-gp inhibitor concurrently with HIV-1 protease inhibitors has been demonstrated in mice by Choo et al. (2000). In the presence of LY-335979, a potent P-gp inhibitor, up to 37-fold increase in the brain concentration of $[^{14}C]$nelfinavir was observed. The plasma level of the labeled drug was only moderately increased suggesting that the increases seen in the brain was due to increased tissue penetration (Choo et al., 2000). If this can be demonstrated in humans, it would be appropriate to add P-gp inhibitors to enhance the availability of HIV-1 protease inhibitors in the brain in the treatment of CNS infections in AIDS patients.

Other approaches (reviewed in Fardel et al., 1996) are also being explored to overcome P-gp mediated drug resistance. These include P-gp specific monoclonal antibody use to block the protein, liposomes to deliver anticancer drugs so that the drugs entirely bypass the P-gp in the lipid bilayer, and nanosphere loaded drugs with a similar rationale. An MDR1-specific antisense oligonucleotide approach is also under investigation to decrease the expression of P-gp mRNA levels (Fardel et al., 1996).

Regardless of the various choices that may become available in the future in circumventing P-gp mediated drug resistance, it is imperative to keep in mind the therapeutic goals when using them, because they could potentiate adverse side effects of the pharmacologic agent in targeted as well as untargeted tissues. For instance, use of P-gp inhibitors along with anticancer agents to treat a brain tumor may inhibit the P-gp

FIGURE 4 The flippase model of P-gp mediated drug transport. The drug intercalates between the phospholipid bilayer of the plasma membrane before interacting with P-gp. Upon interaction with P-gp, the drug is flipped from the inner leaflet to the outer leaflet of the lipid bilayer from where it diffuses to the extracellular space. The flipping process is the fast step, and the entry of the drug from the inner leaflet to the cytosol is the slow step (Johnstone et al., 2000).
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P-gp forms a functional barrier in the BBB and restricts access of various pharmacologic agents to the brain. It is a plasma membrane glycoprotein expressed on the apical surface of endothelial cells that is thought to engage in drug efflux in an ATP-dependent fashion. Although it is not clear how P-gp is able to bind to and expel numerous structurally unrelated compounds, some models have been proposed to explain its mechanism of action. Understanding P-gp’s mechanism of action would enable rational exploitation of its features to allow better drug entry into the brain to treat CNS disease.

While the role of P-gp to keep potentially harmful compounds out of the brain may seem advantageous, it also poses a problem in the pharmacologic treatment of diseases such as brain tumors and brain infections in AIDS patients. The drugs intended to treat diseases affecting the brain are often P-gp substrates; this results in their expulsion even before they have a chance to treat the underlying pathology. Increasing the concentration of the drug to achieve entry into the brain to circumvent P-gp’s action might present problems such as systemic toxicity.

If P-gp inhibitors or reversal agents become available, it might be possible to administer drugs targeted to the brain concurrently with reversal agents. This would overcome the expulsion of many P-gp substrates from the brain and increase the intended therapeutic benefits. The discovery of reversal agents is promising in increasing the bioavailability of various drugs to the brain. However, the development of specific and effective P-gp inhibitors has been hampered by the lack of structural information regarding substrate specificity and binding properties of this protein.

Understanding the structure of P-gp and its mechanism is crucial to the development of P-gp inhibitors and systemic drugs as well. With the help of P-gp reversal agents CNS drugs would achieve better entry into the brain. Similarly, drugs used to treat systemic diseases would also benefit from this knowledge, since these drugs could be developed such that they are better substrates for P-gp and hence are preferentially kept out of the brain thereby reducing neurotoxicity.

However, when P-gp reversal agents do become available for clinical use caution must be exercised in using them, because P-gp is also expressed in tissues other than the brain. Therefore, pharmacologic treatment of diseases using drugs concurrently with P-gp reversal agents must be done after assessing the risks associated with their use.

REFERENCES


