P-GLYCOPROTEIN IN BLOOD BRAIN BARRIER

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ABSTRACT

The endothelial cells of the blood brain barrier prevent the transport of enormous number of substances into the brain. An important component of this barrier is the P-glycoprotein (P-gp). It is an efflux protein that is ATP dependent and thus rightly called the "traffic ATPase" involved in extrusion of compounds, using the energy of ATP hydrolysis to move the variety of structurally unrelated compounds and preventing their accumulation within the brain. Understanding of structure and function of the drug transporter P-gp in the blood brain barrier will pave the way to more tailored and targeted therapies for complex diseases.

Key Words: *P-Glycoprotein, Blood brain barrier, Drug resistance.*

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INTRODUCTION

Blood Brain Barrier

In terms of cellular transport mechanisms, the blood brain barrier (BBB) represents a very important biological barrier between the brain and systemic circulation. This is formed by the capillary network which supplies blood to brain. In fact the capillary endothelium of the brain forms the BBB and there is a complete separation of the brain from the blood through the tight junctions in the capillary endothelium. The BBB functions to restrict almost all the molecules from entering the brain with the exception of those that are either too small or are lipophilic[1]. This property of BBB is very important in order to give protection to the brain from toxic substances as well as helps to keep the internal environment of brain that is the must for proper functioning of the brain.

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The existence of blood brain barrier for the first time was demonstrated in 1880s by Paul Ehrlich when he made use of dyes. Much later in 1960s with the advent of electron microscopy tight junctions in the endothelial cells were identified[2]. Now with recent advances in science a better understanding of the BBB has become possible. It has been established that in the BBB there are specialized endothelial cells that line the capillaries of the brain. Some of the cardinal features of these endothelial cells are the tight junctions, pinocytic vesicles and the absence of fenestrae[3]. Not very long ago the existence of wide number of transporters has been established at BBB. Among these transporters, the most widely studied and the most important one is the P-glycoprotein (P-gp) where the letter P stands for permeability. It was initially expressed in Chinese hamster ovary cells in 1976 and has been designated as 170 kDa protein[4].

P-glycoprotein; the location and structure

P-gp is a phosphorylated glycoprotein which is located on chromosome 7. It contains 27 exons spread over 100 kb. Its existence in the human BBB was detected in endothelial cells in 1989[5]. Later on the existence of this transporter was localized in brain capillary endothelial cells of several species, including monkeys, rats, mice, cattle, and pigs. It is suggested that it provides a protective mechanism in the mammalian BBB and protects the brain from harmful substances. It is now realized this P-gp being an important constituent of BBB is present in abundance on brain capillaries[6]. It is said to be most likely present in the luminal membrane of the brain capillary endothelial cells, although this does not preclude that it can also occur in astrocytes, espe-

cially in certain pathological states[7]. Its existence in bile canaliculi, kidneys, testes and intestine has also been documented[8].

Human P-gp is a single chain of polypeptide comprising 1280 amino acids. These amino acids are arranged in two similar halves and are linked by a linker region. Each half has 6 transmembrane domains which determine substrate specificity. They are essential in terms of transport pathway. Besides this there is a hydrophilic domain which is present on the cytoplasmic side of the cell membrane. It couples the hydrolysis of ATP. The N-glycosylation of P-gp on the first extracellular loop is said to affect the effectiveness of protein. Several kinases phosphorylate the phosphorylation sites on P-gp. This phosphorylation results in alteration of resistance to drugs in some cell lines[9]. Several steps are involved in the transport process, the details of which however still remain unanswered and requires intensive study. For this the structure based models would be helpful that might eventually unravel the mechanism of P-gp export. All the efforts in this regard are so far on the animal models and the efficacy in human brain tissue is lacking.

Functions of P-glycoprotein

P-gp basically functions as an efflux pump. This P-gp is also known as the Multi-Drug Resistance Transporter 1 (MDR1) and by its gene name, ABCB1. It has been suggested that the uptake of the drugs into the brain may be regulated by P-gp. P-gp has an astonishing property of transporting a large list of substrates including vinca alkaloids, epipodophylotoxins, taxanes, anthracyclines[10], cyclosporin A, digoxin[11], and various HIV protease inhibitors[12]. All these substrates vary in physical and chemical properties, yet are identified by the transporter. It is assumed to be contributed to the large molecular weight, increased lipophilicity and an amphipathic nature of compounds but the exact mechanism is yet not known. The drugs which are the weak substrates usually do cross BBB and produce their CNS effects, but somehow the expression and function of the transporter does affect the overall effect[13]. The strong substrates have their effects restricted only to the periphery as they do not cross the BBB[14].

The substrates of P-gp when try to enter endothelial cells from the blood are instantly pumped back into the blood. Thus there is a drastic decrease in the final concentration of the drug in the brain. It is involved in extrusion of compounds, using the energy of ATP hydrolysis to move the solutes across the cellular membranes in all mammalian species[15]. As an efflux pump it can cause a decrease in the cellular accumulation of various drugs including some anticancer drugs, immunosuppressant and protease inhibitors[16,17].

While studying the effects of CNS drugs, it is essential that the concentration of the drug in the brain be determined as well as the transport mechanisms be known. This concentration of drug will then in turn determine the intensity of the drug action. The micro dialysis techniques have proved to be an important approach for this.

Mechanism of P-glycoprotein

There are several theories that help in elaborating the role of P-gp at BBB. It is said that P-gp may be extruding the substrates from lipid bilayer to the extra cellular space[9]. It is further added that it may be transporting substrates from inner leaflet to the outer leaflet of the luminal membrane[18]. Another theory is that it may be detaching the substrates by modifying the membrane composition[9]. An X-ray crystal structure shows that drugs interact with P-gp within the transmembrane regions by fitting into a large flexible binding pocket, which can accommodate several substrate molecules simultaneously. The nucleotide-binding domains of P-gp appear to hydrolyze ATP in an alternating manner; however, it is still not clear whether transport is driven by ATP hydrolysis or ATP binding. The details of the mechanism still need lot more intensive studies.

Role of the P-glycoprotein in drug resistance

On the one hand the P-gp is protecting against the harmful substances and on the other side it does not let the drugs reach their target sites. This problem in encountered while treating brain infections and brain tumors. The drugs are expelled before they have a chance to treat the pathology. Cancer chemotherapies have long witnessed the phenomenon of multi drug resistance[19]. The same has also being encountered with epilepsy, rheumatoid arthritis as well as psychiatric illnesses. The resistance is so much that at times one has to switch over to other therapeutic possibilities. The expression level and functional integrity of this transporter will thus affect the intracellular drug concentrations and thus can play an important role in drug efficacy and toxicity during treatment. The brain expression of MDR1 was first demonstrated by Tishler et al in 1995 where increased expression of the transporter was documented in individuals with resistant epilepsy[20]. Likewise the exact basis for drug resistance in depression is still unknown but in one of the studies the overexpression of P-gp was found to be the cause.

The brain tumors are another example where the expression of P-gp is enhanced and thus contributes towards developing resistance to the drugs that are

used for the said purpose. Moreover most of the drugs used in chemotherapy are the substrates of P-gp which thus get expelled from the brain. Again the entry of anti-viral drugs is limited by P-gp and thus the efficacy of these compounds becomes limited in viral eradication. In all these examples the over expression of multidrug transporters has been reported. However further studies are needed to augment this hypothesis. The better the understanding of structure and function of P-gp, the more is the possibility of combating the problem of drug resistance.

Regulation of P-Glycoprotein

The expression of P-gp is affected under several conditions. It has been observed that on exposure to xenobiotics and hormones in vivo there is upregulation of the P-gp. The expression on the other hand is modulated when exposed to xenobiotics and hormones in vitro. A solid association has been reported between the relapse of sarcoma and the increased expression of MDR1 genes. The outcome of neuroblastoma has a similar correlation. When cell lines are treated with anti-cancer drugs in vitro, the induction of P-gp is reported. This is related to the fact that MDR1 promoter directly responds to cytotoxic agents. Some other agents that have similar actions are verapamil, osmotic shock, low pH, retinoic acid, arsenite, cadmium chloride, steroid hormones, and heat shock. The modulation of P-gp levels appears to be cell and tissue specific as demonstrated by the down regulation of P-gp in leukemia cells by verapamil[21].

Reversal Agents

Inhibiting p-glycoprotein has been an issue ever since P-gp was discovered 35 years ago. Research has shown that thioxanthones have an antitumour activity and these combined with an amine, which is thought to have some P-glycoprotein inhibiting effects could possibly be the answer to chemotherapy drug resistance where p-glycoprotein is present. So far these have not been used therapeutically. 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.

While treating the diseases of CNS like infections in AIDs, a great challenge is encountered. The drugs like HIV-1 protease inhibitors which are the substrates of P-gp are not only extruded by the P-gp but there is also up regulation of this protein and resultant decreased levels of drug in the brain[13]. For combatting this problem the reversal agents are coming up with the hope to block P-gp functions. The reversal agents include the calcium channel blockers, calmodulin antagonists, immunosuppressive agents antibiotics, hormonal analogues and many more. Then P-gp specific monoclonal anti-body blocks the protein and thus the drugs entirely bypass the P-gp in the lipid bilayer. Investigations are being carried out to evaluate the effectiveness of MDR1-specific antisense oligonucleotide in decreasing the expression of P-gp mRNA levels[22].

The use of P-gp inhibitor with HIV-1 protease inhibitor has been employed in mice[23]. This needs to be worked up in humans too. However the dose that will produce significant inhibition of P-gp will also produce undesirable adverse effects. Moreover in order to achieve higher concentration of drug in brain, the increase in the dose of the drug itself will increase the chances of systemic toxicity. No doubt in near future many options may become available in treating P-gp mediated resistance however it has to be borne in minds that they will also be increasing the risk as P-gp is also expressed in tissues other than brain. For instance, use of P-gp inhibitors along with anticancer agents to treat a brain tumor may inhibit the P-gp present in liver and kidney, leading to altered drug metabolism and excretion, as well as increased toxicity. So far the lack of precise picture of structure and function of the P-gp is the main hindrance in the development of effective P-gp inhibitors.

CONCLUSION

P-gp an efflux transporter is able to bind and expel clinically significant compounds however there is a complex mechanism underlying this activity across the BBB. The understanding, however, still remains far from complete. The better understanding of the whole process will prove a landmark in choosing the drugs that will be effectively producing their effects in CNS in spite of the action of this transporter. Either these drugs will be by passing P-gp, or they will not be the substrates of P-gp or will be the inhibitors of the transporter. The options will keep on increasing with the better understanding of the subject. It therefore necessitates the requirement of more efforts in this direction to combat the problems associated with it. This will be of tremendous help in directing future drug development research.

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