Research Article

Permeability Glycoprotein : A Boon Or Bane ?

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ABSTRACT

P glycoprotein (P gp) is a 170kD protein product of the ABCB1 gene located on chromosome 7q21. It consists of two membrane bound domains and two cytoplasmic nucleotide binding domains. It functions as an efflux pump located on the apical epithelium of intestine, pancreas, gut and endothelial lining of various tissues including the blood brain barrier. Apart from protecting the body from the toxic effects of xenobiotics, it also plays a role in determining the bioavailability and distribution of drugs. The vacuum model and the flippase model have been proposed as the mechanism of action. The broad substrate specify shared between P gp and cyp3A4 has resulted in assumption that they probably work in concert with each other. Over the recent years, it has been postulated that genetic polymorphism in the expression of MDR1 gene regulates the response to various drugs. Therefore multiple drug resistance (MDR) may be genetically determined. The susceptibility of individuals to diseases such as ulcerative colitis and cancer is also determined by the expression of this unique pump. It has been found to be responsible for 30-40% of treatment failure in epilepsy. Recently various modulators of P gp are being exploited in order to overcome drug resistance. Unlike the first two generations, the third generation modulators like tariquidar are highly selective for P gp and do not interact with cytP450.Thus P gp can prove to be a vital tool in assessing the genetic susceptibility of an individual to disease and drug resistance and thus tailor therapy to them.

Keywords: P glycoprotein, multi drug resistance , modulators, diseases

INTRODUCTION

The ABC super family of membrane proteins, classified into 7 sub families, is one of the most extensively studied family till date. The Permeability glycoprotein (P gp) or cluster of differentiation 243 protein is a member of ATP Binding Cassette, sub family B and member 1 (abbreviated as ABCB1). It constitutes one of the chief efflux pumps in the body. Since its discovery in 1976 by Victor Ling in colchicine resistant Chinese hamster ovary cells, it has been found to play an imperative role in preventing the body from the brunt of xenobiotics. Thus it is also known as the gatekeeper protein of the body. It is encoded by the ABCB1 gene located on chromosome 7q21 and consists of 28 exons. Due to its broad substrate specificity, it exhibits a wide range of drug interactions which has resulted in alteration of response to drugs. It has become one of the leading targets to surmount resistance in cancer cells.

Location and Function

P gp is known to be extensively distributed throughout the body. However ,with the help of monoclonal antibody MRK16, it was found to be predominantly located on the epithelial lining of the gastrointestinal tract such as in pancreas, colon, jejunum, liver cells, and in the Proximal convoluted tubule of the kidney thus emphasising its fundamental role in preventing the body from the foreign chemical substances such as toxins and drugs[1]. Being situated on endothelial lining of the various barriers such as the blood brain barrier, blood testes barrier, it also limits the entry of certain drugs into these sensitive areas. Due to its location on canalicular membrane of hepatocytes, it also promotes drug elimination through bile. It also disseminates the endogenous steroid hormones from the adrenal gland through its efflux action. Multi drug resistance in cancer cells has been attributed to the over expression of this efflux pump which results in the alteration of the pharmacokinetic as well as the pharmacodynamics parameters of the drugs.

Structure

P gp being a member of the ATP Binding Cassette family (ABC) constitutes two membrane spanning domains (MSD) and two nucleotide binding domain (NBD). It forms an internal cavity of approximately 3000 Angstroms with a separation of 30 Angstroms between the two NBDs[2]. The MSD spans the membrane in the form of six transmembrane α helices. The NBD possesses constitutive ATPase activity and is responsible for the binding and hydrolysing of the ATP. It is funnel shaped and narrower on the cytoplasmic side.

MATERIALS AND METHODS

Mechanism of Action

Various mechanisms have been postulated for the mechanism of action of P gp. The most universally accepted model is that of its action in a pump manner. Following the binding of the drug to NBD, the catalysis of ATP takes place, and it pumps the drug from inside to out. However whether it requires one ATP molecule or two is still conflicted. According to an article by Sharom, this particular transporter may work as a vacuum to expel the drugs out or may act in a flippase manner by just flipping

the drug from the inner leaflet to outer membrane leaflet.[3] Since the binding of the drug takes place with the help of hydrogen bonds and Vander Waal, it is more likely that the drug is induced to fit into the pocket. This may be the reason as to why P gp possesses the capacity to interact with innumerable structurally unrelated drugs. Some of the substrates have been mentioned in the following table.

Table 1: Substrates of P gp

S. No.	Drug Classification	Drugs
1.	Anti Cancer	Paclitaxel
		Etoposide
		Vincristine
2.	Antiepileptics	Phenytoin
		Phenobarbital
3.	Opioids	Morphine
		Loperamide
4.	HIV Protease Inhibitors	Indinavir
		Saquinavir
5.	Antifungal	Itraconazole
6..	Calcium Channel Blockers	Diltiazem
		Verapamil

Relation Between P GP and Cytochrome 3A4 (Cyp3A4)

It has long been known that Cyp3A4, one of the most prominent oxidative cytochrome P450 enzymes plays a major role in first pass metabolism. Since it is also located in the small bowel enterocytes and shares a broad substrate specificity which overlaps with that of P gp, it was suggested that the P gp and Cyp3A4 work in a concerted manner in order to regulate the bioavailability of drugs.[4] In the enterocyte, the P gp is primarily located on the apical surface whereas the cytochrome enzyme is located on the sarcoplasmic reticulum.[5] The P gp works in such a way that it keeps the intracellular concentration of a drug within the range of Cyp3A4. It has been suggested that it may even extend the duration of absorption, thereby increasing the extent of exposure to metabolism by cyp3A4.[6]

However, since this transporter is an active transporter, it gets saturated in cases of quickly absorbed drugs when given in high doses. On a molecular level, the pregnane X receptor (PXR) co regulates the genes for both cyp3A4 as well as P gp.

P GP and Diseases

It has already been established that this transporter is an indispensable asset of our body for protection from toxins. So it also implies that a dysfunctional P gp may result in occurrence of certain diseases. Such theories have been put forward, and the role of P gp in the occurrence of neurodegenerative diseases such as Parkinsons and Alzhiemers has been discussed[7]. It has been reported that mdr1a (murine multiple drug resistance gene) knockout mice are susceptible to develop inflammation comparable to that of human inflammatory bowel disease.[8] It has also been implicated in diseases such as Parkinson and refractory seizures[9] as well as in the pathogenesis of drug refractory epilepsy.[10]

Role in Drug Therapy and Drug Interactions

As quoted by the Agency for Healthcare Research and Quality in 2008, there are more than 2.7 million drug related adverse events per year[11]. This is primarily because polypharmacy has become the trend in medical practice. Thus an understanding of the transporters becomes the need of the hour. Plenty of drugs may serve to be the substrate of P gp and cyp3A4 and may require dose adjustment in order to avoid failure of therapy or drug toxicity. The P gp knock out and control mice models have been used to collect data on the drug interactions with P gp. Thus it will not be wrong to say that drug transporters play a crucial role in determining the response to therapy as they control the bioavailability of the drug as well as the entry of the drugs into tissues. One of the oldest interactions that has been studied is that between digoxin and quinidine when it was found that quinidine increased the levels of digoxin in plasma. These kinds of interactions can have numerous clinical implications and thus a thorough knowledge of the substrates and inhibitors is more or less required in clinical practice. However, apart from interactions, polymorphism in the expression of P glycoprotein plays an inevitable role in governing the overall plasma levels of drugs such as protease inhibitors and hence the magnitude of CD 4+ cell count recovery.[12]

Role in Multi Drug Resistance (MDR)

Multi drug resistance can be defined as a phenomenon in which cells display a lack of response to drugs which are structurally unrelated. After extensive research, a common mechanism for the occurrence of this kind of resistance was hypothesised, which was an over expression of the drug transporters. Recently, P gp over expression in cancer cells has ignited a lot of potential in research area with respect to resistance. In order to elucidate the role of this transporter in resistance such as cancer cells, certain criteria need to be met. An over expression of this transporter in the resistant cells, along with correlation of the expression to the degree of resistance and inhibition of this transporter resulting in reversal of the resistance are some of them that need to be demonstrated.[13] Non responsiveness to antiepileptic drugs has also been attributed to the genetic variation in the expression of P gp as it can decrease the entry of the amount of drug into the epileptic focus.[14] This is a crucial as about 30% individuals are affected by this resistance.[15]

Role in Drug Resistant Epilepsy

About 30 % of the epileptic patients are known to be resistant to pharmacotherapy of epilepsy. Though the exact mechanism of it still remains unknown, one possibility is an amplified expression of P gp which results in a decreased level of the drugs entering into the brain.

Persistent sub therapeutic levels of antiepileptics have been co related with the increased expression of this particular transporter. It has been found to be involved to result in a plodding loss of response to drugs such as phenytoin.[16] Marchi performed a pilot study to illustrate that the failure of the drug oxcarbazepine is primarily due to the increased expression of P gp and not merely the alteration in drug metabolism.[17] Thus impeding this efflux pump, by virtue of use of P gp inhibitors can lead to an increase penetration of the drug across the blood brain barrier. One of the other strategies, to overcome the hindrance posed by P gp is modulating the pathways that lead to its up regulation. One such pathways discussed previously in a review by Anika, suggests that an increase in glutamate during seizure which further acts on N-methyl D aspartate receptor (NMDA) results in upsurge of P gp expression.[18] This, along with cyclooxygenase enzyme 2 (cox-2), one of the downstream targets of glutamate plays an imperative role in regulating P gp expression. Thus they can be paramount targets of newer drugs to overcome the clinical problem of drug resistant epilepsy.

Role in Cancer Chemotherapy

It has been known that the response to cancer chemotherapy is highly limited due to the presence of drug transporters. P gp and other transporters such as BCRP (Breast Cancer Resistant Protein), LRP (Lung resistance-related protein) play an inevitable role in this lack of response to therapy.[19]

This transporter may be expressed in a constitutive manner in cancers such as colorectal cancer or may be acquired in malignancies such as leukemias.[20] Resistance to common anti cancer drugs such as doxorubicin in small cell lung cancer has long been attributed to P gp, which has further been reverted by verapamil.[21]

It has even been suggested that addition of a platinum compound such as cisplatin may result in inhibition of this transporter and it may prove to be beneficial for the patient.[22]

Clinical pharmacology of drugs such as etoposide, paclitaxel, and vinblastine can be modulated with the co administration of P gp inhibitors in order to attain remission in drug resistant cases.

P GP Inhibitors

The P gp inhibitors have been classified into 3 generations.

First Generation

The first generation inhibitors such as verapamil, cyclosporine, quinidine were not initially developed with the purpose of actually inhibiting P gp. They were highly diverse and had a broad spectrum of activity. They were further found to be substrates of P gp and were eventually found to act as competitive inhibitors. However being non selective and less potent, they could inhibit P gp only at a very high dose. Therefore, could not be clinically used for this purpose.

Second Generation

The second generation agents such as dexverapamil, Valspodar and Biricodar were found to be less toxic as compared to the first but had numerous interactions due to the overlap in substrate activity with cyp3A4. Thus the trials with these compounds were unsatisfactory.

Third Generation

Inhibitors of P gp such as Tariquidar (XR9576) Zosuquidar (LY335979) and Laniquidar (R101933) selectively and potentially inhibit P gp. Being highly potent, Tariquidar can inhibit P gp even in nanomolar concentrations.[23]

CONCLUSION

Even though with the newly emerging drug delivery systems such as nanoparticles, the problem of drug resistance is being tried to overcome, P gp remains a crucial target for the researchers. In these past two decades, a lot has been understood about this transporter at the molecular level; however its clinical implications still evade the mind of a physician on a daily basis. Thus, more attention needs to be given to this transporter when deciding a regime for an individual.

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