EFFLUX TRANSPORTERS: ROLE IN DRUG BIOAVAILABILITY, EFFLUX AND RESISTANCE

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ABSTRACT

ABC transporter proteins are the receptors which regulate the entry of lipophilic drugs and xenobiotics through cell membrane. It does not allow xenobiotics to accumulate at site of action. So there is need to study about ABC transporters in detail so that we may enhance the transportation of drug by inhibiting these transporter receptor.

KEYWORDS: Xenobiotics, ABC transporters, P-glycoprotein.

INTRODUCTION

ABC transporter is a family of transport receptor proteins mainly P-glycoproteins which regulates the efflux of any xenobiotic across cell membrane. P-glycoprotein also interferes with drug bioavailability and disposition, including absorption, distribution, metabolism and excretion, influencing pharmacokinetic and pharmacodynamic of drugs. Modulation of P-gp may help the efficacy of the treatment of several diseases and can explain some adverse central nervous system effects induced by drugs after intravenous administration and the poor response of oral administration in patients. Alteration in P-gp expression or function has been associated with several diseases susceptibility in humans and animals. Hence there is a need to study P-gp efflux in detail.

Literature states that the main physiological role of these proteins is to protect the organism from xenobiotics. It allows the entry of drug such as lipophilic drugs in brain but after saturation they identify these drug molecule as foreign body (xenobiotics) and does not allow them to accumulate at site of action. Due to this mechanism the required amount of drug is not obtained for pharmacological action. Hence, there is a need to study about ABC
transporters in detail so that we may enhance the transport of drug by inhibiting these transporter receptors. Another drawback of these efflux proteins is that they cause resistance of drugs for instance verapamil, cyclosporine A, trans- flupenthixol. This mechanism is known to be as multi drug resistance.

In this article we are mainly concerned with ATP binding cassette transporters which influence transport of drugs across endothelial cell membrane which causes low bioavailability.

**ABC FAMILY**

ABC Transporters are multi-domain integral membrane proteins that use the energy of ATP hydrolysis to translocate solutes across cellular membrane in all mammalian species.\[^{12}\]

ABC Transporters form one of the largest of all protein families and are central to many important by medical phenomena such as resistance to chemotherapeutics. ABC Transporters are increasingly recognized to be important for drug disposition and to response.\[^{12}\] The human genome contains 48 ABC genes, of which 60 have a known function and 14 are associated with a defined human disease. These functional transporter proteins are usually comprised of two NBFs (nucleotide binding folds) and two trans-membrane (TM) domains. The physiological functions of ABC transporter include the transport of lipids, bile acids, peptides and toxic compounds. Therefore these efflux transporters have received considerable attention as they detrimentally alter pharmacokinetics and pharmacodynamics of administered drugs.\[^{9}\]

These efflux proteins are member of ATP binding cassettes superfamily includes ABCB1/MDR1 P-gp, ABCC2/MRP2 and more recently ABCG2/BCRP.

**P-GLYCOPROTEINS**

P-glycoprotein was first described in 1976, by JULIANO & LING in Chinese hamster ovary cells selected in culture for colchicines resistance (Marzolini et al, 2004) and is the most studied ABC drug efflux transporter to date.

P-glycoprotein (P-gp) is a 170 K dalton transmembrane protein encoded by MDR1 multi drug resistant gene containing 27 axons located on the chromosome 7 and has N- and C- terminals that transports a broad range of chemically diverse hydrophobic compounds, including chemotherapeutics, conferring multi drug resistance on cells. In humans P-gp is
encoded by two MDR [MDR1 and MDR2] genes (Callen et al, 1987; chin et al 1989). Three genes have been identified in rodents [mdr1a, mdr1b and mdr2] (Gross et al., 1986; Ueda et al., 1986).

P-gp belongs to the family of ATP – binding cassette (ABC) transporters, it is comprised of two homologous halves, each containing 6 transmembrane α-helices and an ATP binding site characterise by an “ABC signature” element, in addition to walker A and B sequence motives. The α-helices form a pore-like structure allowing the passage of a wide range of hydrophobic substrate against their concentration gradient, governed by ATP fuelled conformational changes of the proteins.\(^3\)

The most striking property of ABCB1/ P-gp includes its broad substrate specificity and transports large number of structurally diverse drugs. Hydrophobicity, Planar aromatic rings and the presence of tertiary amino groups favour substrate interaction with P-gp and recently pharmacophore models of P-gp substrate affinity have been proposed.

**MECHANISM OF ACTION OF P-glycoprotein**

Transport can be broken down into several steps: entry of substrates into the binding pocket within the cytoplasmic leaflet, conformational changes in P-gp driven by ATP binding/hydrolysis, and release of drug to either the outer leaflet or the extracellular aqueous phase. Many different approaches had been carried out to explain mechanism of P-gp which involves experimental and model based approaches. Various models which have been proposed to depict the mechanism of efflux by P-gp includes:
1) **The classical model**: It 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 (Borst and Schinkel, 1997).

2) **The hydrophobic vacuum model**: It suggests that P-gp binds directly to the substrate on the plasma membrane and recognises these as foreign, as consequence pumps it out of the cell (Fardel et al, 1996).

3) **The pH model**: It proposes P-gp secondary role in drug transport that it alters the intracellular pH or the membrane potential by functioning as a protein or chloride pump, And thus reduces the accumulation of cationic lipophillic drugs (Fardel et al, 1996).

4) **The flippase model**: This model proposed that P-gp encounter the drug in internal leaflet and flips out the drug in outer leaflet against concentration gradient accompanied by ATP hydrolysis. Currently this is the most favoured model.

**Flippase model**: This model suggests that when a drug molecule interacts with membrane it is accessed as a substrate by the core of TMDs of the lipid bilayer. Hence, it flips out the molecule to the outer membrane. This substrate intercalates between the phospholipid layers prior to P-gp interaction, and then interacts with P-gp binding site.

After drug interaction with P-gp the coordinated domains of P-gp namely TMDs 6 and 12 and the nucleotide binding domains performs the drug efflux mechanism. The residues of both the domains forms the drug binding pocket following ATP hydrolysis at alternating sites, leading to conformational changes in the TMDs (for drug binding pocket formation N- and C- terminals of TMDs are required). This results in reduced affinity of TMDs 6 and 12 with drug resulting drug efflux (Loo and Clarke, 1999).

**LOCATION OF P-glycoprotein**

MDR P-gp is present in many human tissues such as liver, Kidney, Intestines and adrenal glands as well as in blood-tissue barriers including the placenta, testis capillaries and brain capillaries.
In Liver, P-gp is found exclusively on the biliary canalicular front of hepatocytes and on apical surface of epithelial cells in small biliary ductules. In Pancreas, P-gp is found on the apical surface of the epithelial cells of small ductules but not larger pancreatic duct. In Kidney, P-gp is found concentrated on the apical surface of epithelial cells of the proximal tubules. Both Colon and Jejunum show high levels of P-gp on the apical surfaces of superficial columnar epithelial cells. Adrenal gland shows high levels of P-gp, diffusely distributed on the surface of cells in both the cortex and medulla. Its expression is also detected in specialized epithelial cells with secretory or excretory functions, trophoblasts in the placenta, and on endothelial cells of capillary blood vessels at blood-tissue barrier sites.

**EFFECT OF ABC TRANSPORTERS ON BIOAVAILABILITY**

**Bioavailability** is the rate and extent of the given xenobiotic (drug) that reaches systemic circulation with time. Bioavailability is mainly influenced by transport mechanism of efflux (e.g. P-glycoprotein) i.e permeability of drug. Now a days there is a need to step out to increase the amount of drug that will reach the site of action to get pharmacological action. As after release of drug from formulation the next rate limiting step is transport of drug which is regulated by ABC Transporters.

Oral bioavailability of drugs was initially thought to be a function of absorption and phase-I metabolism. However, with the advancement in pharmacokinetics, it has become apparent that active efflux via P-gp in the small intestine is a major contributor to poor absorption and consequently low bioavailability. P-gp appears to influence the peak concentration of orally administered drugs in the systemic circulation as a drug molecule that is a P-gp substrate crosses an epithelial cell membrane by simple diffusion. After diffusion it might continue to
Diffuse along the concentration gradient up to mesenteric circulation. But sometimes it is removed back to the lumen by the efflux transporters and results in decrease bioavailability.

**DISTRIBUTION:** However all the above factors are major contributors to drug bioavailability, along these drug distribution is also an important factor in drug deposition, especially in brain and fetus. Brain anatomically expresses barrier to xenobiotics which is Blood Brain Barrier (BBB). BBB expresses P-gp to different extents prohibiting the accumulation of toxins and P-gp substrates from being distributed to brain. So P-gp in BBB limits the penetration of CNS targeted drugs. One of the best example describing the role of P-gp in limiting CNS penetration of drugs is the Anti-diarrheal drug loperamide which is a potent opioid as it is a P-gp substrate so is unable to cross the BBB.

**Table: List of ABC transporters affecting drug availability in brain**

<table>
<thead>
<tr>
<th>ABC Transporters</th>
<th>Substrates</th>
</tr>
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</table>
| **P-glycoprotein (ABCB1)** | **Anticancer drugs** e.g., Doxorubicine, daunorubicine, vinblastine, vincristine, etoposide, teniposide, paclitaxel, methotrexate  
**Immunosuppressive agents** e.g., Cyclosporin A  
**Corticoids** e.g. cortisol Dexamethasone, hydrocortisone, corticosterone, aldosterone  
**HIV protease inhibitors** e.g., Amprenavir, indinavir, saquinavir  
**Cytokines** e.g., IL-2, IL-4, IFN-γ  
**Antidiarrheal agents** e.g., Loperamide  
**Anthelminthic agents** e.g., Ivermectin, abamectin  
**Anti-gout agents** e.g., Colchicines  
**Histamine H2-receptor antagonists** e.g., Cimetidine  
**Calcium channel blockers** e.g., Verapamil  
**Antiemetics** e.g., Domperidone, ondansetron  
**Cardiac glycosides** e.g., Digoxin  
**Diagnostic (fluorescent) dyes** e.g., Rhodamine-123  
**Antidepressants** e.g., Amitryptiline, nortryptiline, doxepin, venlafaxine, paroxetine  
**Antibiotics** e.g. Erythromycin, valinomycin, tetracyclines, Fluoroquinolines |
| **2)MRP1 (ABCC1) & MRP2 (ABCC2)** | **Anticancer drugs** e.g., Etoposide, teniposide, vincristine, doxorubicine, daunorubicine, methotrexate;  
**Leukotrienes:** C4 (LTC4), D4, E4;  
Various glutathione, glucuronide, and sulfate conjugates, but also unconjugated compounds (e.g., fluorescein) |
| **3)MRP3 (ABCC3)** | Organic anion transporter with considerable overlap in drug substrates with MRP1 and MRP2 |
| **4)MRP4 (ABCC4)** | **Anticancer drugs** such as methotrexate, thioguanine  
6-mercaptopurine |
ATP binding cassette (ABC) transporters are a family of transporter proteins that contribute to drug resistance via ATP dependent drug efflux pump mainly by P-glycoprotein(P-gp) encoded by the MDR1 gene, is an ABC transporter normally involved in the excretion of toxins from cells, which is energy dependent mechanism driven by ATP hydrolysis and effect bioavailability of drugs as follows:

1. Over expression of ABC efflux transporters (P-glycoprotein) at baseline of tumours such as colon, kidney, cancers, breast cancer, BBB resulting in pharmaco resistance to therapeutic medication hence decrease bioavailability.

2. Both cytochrome P450 (CYP) 3A isoenzymes and efflux proteins, belonging to the ATP-binding cassette (ABC) transporter family (eg, ABCB1/P-glycoprotein, P-gp). Recently, similarities between the tissue distribution and gene regulation of CYP3 A4 and P-gp, generated the hypothesis that CYP3A4 and P-gp have a complementary function and thus form a barrier to drug absorption and metabolism.[4]

**ROLE OF ABC IN BLOOD BRAIN BARRIER**

The ABC efflux transporter P-glycoprotein (P-gp) has been demonstrated as a key element of the BBB that can actively transport a huge variety of lipophilic drugs out of the brain capillary endothelial cells that form the BBB. In addition to P-gp, other ABC efflux transporters such as members of the multidrug resistance protein (MRP) family and breast cancer resistance protein (BCRP) seem to contribute to BBB function. Consequences of ABC efflux transporters in the BBB include minimizing or avoiding neurotoxic adverse effects of drugs that otherwise would penetrate into the brain. However, ABC efflux transporters may also limit the central distribution of drugs that are beneficial to treat CNS diseases.

Drug uptake into the brain is dependent on a variety of factors, including the physical barrier presented by the BBB and the blood-CSF barrier (BCSFB) and the affinity of the substrate for specific transport systems located at both of these interfaces.[11,12] It is the aggregate effect
of these factors that ultimately determines the total brain exposure, and thus pharmacological efficacy, of a drug or drug candidate. In general many of lipid soluble drugs are more readily will tend to partition into brain tissue but a very significant number of lipid soluble molecules have lower brain permeability. These lipid molecules are substrates for the ABC efflux transporters in the BBB and CSF and the activity of these transporters very efficiently removes the drug from the CNS, thus limits the brain uptake.

**BRAIN**

![Diagram of ABC transporters in the brain capillary endothelial cell](image)

<table>
<thead>
<tr>
<th>ABC Transporters</th>
<th>Substrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) P-glycoprotein (ABCB1)</td>
<td><strong>Cytokines</strong> e.g., IL-2, IL-4, IFN-α</td>
</tr>
<tr>
<td>2) MRP1 (ABCC1)</td>
<td>Various glutathione, glucuronide, and sulfate conjugates, but also unconjugated compounds (e.g., fluorescein)</td>
</tr>
<tr>
<td>3) MRP2 (ABCC2)</td>
<td>Organic anion transporter with considerable overlap in drug substrates with MRP1 and MRP2</td>
</tr>
<tr>
<td>4) MRP3 (ABCC3)</td>
<td>Organic anion transporter with considerable overlap in drug substrates with MRP1 and MRP2</td>
</tr>
<tr>
<td>5) MRP4 (ABCC4)</td>
<td>6-mercaptopurine</td>
</tr>
<tr>
<td>6) MRP5 (ABCC5)</td>
<td>cGMP, cAMP, 6-mercaptopurine, thioguanine, fluorescein</td>
</tr>
<tr>
<td>7) MRP6 (ABCC6)</td>
<td>BQ-123 (an anionic cyclopentapeptide and endothelin receptor antagonist)</td>
</tr>
<tr>
<td>8) BCRP (ABCG2)</td>
<td>Sprazosin</td>
</tr>
</tbody>
</table>
ROLE OF ABC IN MDR RESISTANCE

Also Multidrug resistance is a phenomenon whereby infected cells in vitro that have been exposed to one cytotoxic agent develop cross-resistance to a range of structurally and functionally unrelated compounds. Multidrug resistance is, in part, the result of an increased expression of efflux proteins such as ABCB1/MDR1 P-glycoprotein, which is a member of ATP binding cassette (ABC) superfamily of transporters.[4] These efflux proteins limits the intracellular exposure to xenobiotics by pumping agent out of the cell. ABC –transporters are also expressed in normal human tissues including gastrointestinal tract, Placenta, Brain, Liver, Lung, Kidney, Feces, Testes etc.[9]

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Conventional name</th>
<th>Location</th>
<th>Resistant to drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCB1</td>
<td>Multidrug resistant gene 1 P-glycoprotein (MDR 1 P-gp)</td>
<td>a).Intestinal epithelium b).Duodenal</td>
<td>Paclitaxel Digoxin</td>
</tr>
<tr>
<td>ABCC2</td>
<td>Multidrug resistant associated protein 2 (MRP2)</td>
<td>Liver, it regulates bile excretion mainly, deficiency causes hyperbilirubinemia</td>
<td>Antibiotics ACE inhibitors</td>
</tr>
<tr>
<td>ABCC3</td>
<td>Multidrug resistant associated protein 3 (MRP3)</td>
<td>-</td>
<td>Indomethain probinacid</td>
</tr>
<tr>
<td>ABCG2</td>
<td>Breast cancer resistance protein (BCRP)</td>
<td>Breast cancer cells</td>
<td>Doxorubicin Anthracyclins</td>
</tr>
</tbody>
</table>

APPROACHES FOR ENHANCING BIOAVAILABILITY

ORAL: There are many approaches to enhance the intestinal absorption of poorly absorbed drugs. These approaches include the use of absorption enhancers, protease inhibitors (which are only effective for peptides and protein drugs), prodrugs and permeability-enhancing dosage forms. Furthermore, the application of P-gp inhibitors in improving peroral drug delivery has gained special interest. Here is a trial demonstrating P-gp inhibition effect on bioavailability.

Literature has states that P-gp plays an important role in limiting the bioavailability of oral drugs. Which can be demonstrated by an example of cyclosporine, as its bioavailability was limited by P-gp efflux pump for instance oral bioavailability of cyclosporine in healthy human volunteers increased from 0.293 to 0.651 upon co-administration of ketoconazole which is a P-gp inhibitor.[16] Hence approach to increase bioavailability is the inhibition of P-gp efflux pump. Which may be either by natural or synthetic sources.
BRAIN

Inhibition of Efflux Mechanism (Atp-Binding Cassette Transporters)

As mentioned previously, the BBB contains several ABC transporters, which expel a multiplicity of drugs from the CNS. Two strategies have emerged for avoiding the activity of these efflux transporters: either by developing specific inhibitors for the efflux transporters, thus giving their substrates a greater access to the CNS, or by attempting to design analogues of drugs with known efficacy but with poor BBB penetration due to ABC transporter activity, which will no longer have a reactivity with the efflux transporters. For both of these strategies to be really effective, it requires a detailed knowledge of the structure-activity relationships (SAR) of the ABC efflux transport mechanisms. This detailed information is proving difficult to obtain. ABC transporters do not interact with their substrates and inhibitors in a classic enzyme-substrate/lock and key manner and therefore standard Menten-Michaelis kinetics cannot be readily applied to their several inhibitors, both competitive and noncompetitive, have been developed to modulate the activity of the major ABC transporters P-gp, MRP, and BCRP. These are shown in following table:

Table: Inhibitors of ABC Transporters that Are Expressed at the Blood-Brain Barrier

<table>
<thead>
<tr>
<th>ABC Transporters</th>
<th>Inhibitors</th>
</tr>
</thead>
</table>
| 1) P-glycoprotein (ABCB1) | **1st Generation** e.g., verapamil, cyclosporine A, quinidine, quinine, amiodarone, detergents such as cremophore EL  
**2nd Generation** e.g., PSC-833 (valspodar), GF120918 (elacridar), VX-710 (biricodar), dexverapamil  
**3rd Generation** e.g., OC 144-093 (ONT-093), LY335979 (zosuquidar), XR9576 (tariquidar), R101933 (laniquidar), GF120918 |
| 2) MRP1 (ABCC1) | Sulfinpyrazone, probenecid, MK571, LTC4, **some P-gp inhibitors** (e.g., cyclosporine A, verapamil, and (PSC 833) |
| 3) MRP2 (ABCC2) | Sulfinpyrazone, probenecid, MK571, LTC4, **some P-gp inhibitors** (e.g., cyclosporine A, verapamil, PSC 833) |
| 4) MRP3 (ABCC3) | Classical **organic anion transport inhibitors** such as sulfinpyrazone, indomethacin, and probenecid |
| 5) MRP4 (ABCC4) |  |
| 6) MRP5 (ABCC5) | Probenecid and **phosphodiesterase inhibitors** such as trequensin or sildenafil |
| 7) MRP6 (ABCC6) |  |
| 8) BCRP (ABCG2) | GF120918 (inhibits also P-gp), fumitremorgin C (FTC) and FTC analogues such as Ko132 and Ko134, CI1033 |
In general, efflux inhibitors can be divided in small molecule inhibitors (SMIs) and polymeric inhibitors. First generation SMIs are pharmacological active compounds such as quinine and verapamil that are used in the clinical indications and which are found to inhibit efflux pumps. Second and third generation SMIs are PSC833, GF120918 or KR30031 have been developed to exclusively inhibit efflux pumps and to avoid further pharmacological interactions. The polymeric inhibitors are of two types natural and synthetic polymer such as shown bellow in the table:

<table>
<thead>
<tr>
<th>S.no.</th>
<th>NATURAL</th>
<th>S.no</th>
<th>SYNTHETIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Anionic gums</td>
<td>1.</td>
<td>Polyethylene Glycol</td>
</tr>
<tr>
<td>2.</td>
<td>Sodium alginate</td>
<td>2.</td>
<td>PEG based detergent</td>
</tr>
<tr>
<td>3.</td>
<td></td>
<td>3.</td>
<td>Poloxamers</td>
</tr>
<tr>
<td>4.</td>
<td></td>
<td>4.</td>
<td>Dendrimers</td>
</tr>
<tr>
<td>5.</td>
<td></td>
<td>5.</td>
<td>Thiomers</td>
</tr>
</tbody>
</table>

**APPROACH TO DECREASE MDR**

Approaches (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, liposome to deliver anticancer drugs are 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).

**MULTIDRUG QUANT ASSAY KIT**

- The kit provides a fast, simple and reliable method to measure the activity of P-glycoprotein (P-gp) and MRP1, simultaneously.
- It was developed as a flow cytometric assay, where intracellular calcein fluorescence is measured after incubating the cells with the acetoxymethylester form of fluorescent calcein (calcein-AM) in the presence and absence of inhibitors of P-gp and MRP1.
- Intracellular fluorescence intensities obtained with or without inhibitors are used for calculation of MDR activity factor (MAF) values, which are the quantitative measures of transport activity of P-gp and MRP1.

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