The Pharmacology of Drug Transporters

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How drugs cross cellular membranes

Significance of drug transporters in the drug response

- Influx
- Efflux
- Plasma/Tissue drug concentration
- Drug distribution
- Drug efficacy
- Drug toxicity
- Tissue expression
- Expression levels
- Activity
- Polymorphisms
- Inhibitors
Normal endogenous function of transporter proteins

Dietary and Environmental Toxins

Nutrients, e.g. Amino Acids

Influx/Uptake transporters

Essential Metabolites, e.g. glucose

EXCRETION/DTOXIFICATION

Signaling Molecules, e.g. hormones

Efflux transporters

Normal Cell Function

And Metabolism

Carrier-mediated drug transport

• Cargo forms complex with transporter
• Transport is subject to saturability
• Transport can be reversible (depending on concentration gradient)
• Rate of transport determined by Michaelis-Menten kinetics
• Transport can be inhibited by other compounds

Different mechanisms of carrier-mediated drug transport

Passive Transport

Facilitated Diffusion

Primary Active Transport

Secondary Active Transport

Immediate gradient of substrate

Transported substrate
Influx and Efflux transporters co-operate to promote vectorial transport of drugs across epithelial/endothelial barriers - absorption/cellular uptake/excretion

Epithelial/Endothelial barrier

Influx Transporter

Efflux Transporter

Drug 1

Drug 2

Blood/Bile

Blood/Urine

Blood/CNS

Selective expression of transporters promotes tissue-specific drug uptake and barrier functions

Tissue 1

Drug efficiently taken up into Tissue 1

Drug Response

Tissue 2

Drug not a substrate for influx transporter expressed in Tissue 2 - No drug uptake

Tissue 3

Efflux transporters form a “drug barrier” by exporting any drug taken up by influx transporters

Role of drug transporters in Pharmacokinetics

Intestinal epithelia - ABSORPTION/EXCRETION

Target tissues - SELECTIVE DRUG UPTAKE/DISTRIBUTION

Liver epithelium - HEPATIC UPTAKE/METABOLISM/ELIMINATION

Kidney epithelium - CLEARANCE/ELIMINATION

CNS endothelium - BLOOD BRAIN BARRIER
Role of drug transporters in mediating adverse drug reactions

Example 1: Uptake and/or Efflux in LIVER/KIDNEY
- Clearance/Plasma Conc.
- Toxicity

Example 2: Uptake and/or Efflux in toxicological target organ
- e.g. Brain
- Cellular Conc./Toxicity

Example 3: Drug inhibits transport of endogenous transporter substrates
- Plasma/Cell Conc. of substrate/Toxicity

Example 4: Uptake and/or Efflux in toxicological target organ e.g.
- Brain
- Cellular Conc./Toxicity

Example 5: Drug inhibits transport of endogenous transporter substrates
- Plasma/Cell Conc. of substrate/Toxicity

Drug Transporters as mediators of Drug-Drug interactions

Drug #1 is specifically taken up into tissue via a specific drug transporter

Drug #2 binds transporter and prevents transport of Drug #1

Plasma concentration of Drug #1

Drug #2: Substrate or inhibitor of Drug transporter

Reduced efficacy of Drug #1 due to decreased uptake

Toxicity

Major classes of transporter proteins implicated in drug transport

Drug Transporters

- Solute Carrier (SLC) Superfamily
- ATP-Binding Cassette (ABC) Superfamily

4 most important sub-families

- OATP: Organic Anion Transporting Polypeptides
- OAT: Organic Anion Transporters
- OCT: Organic Cation Transporters
- MATE: Multi drug and toxin/excretion transporters

3 most important sub-families

- P-gp/MRP: P-glycoprotein/ Multidrug resistance
- BCRP: Breast Cancer Resistance Protein
- MRP: Multidrug resistance proteins
A. OAT: Organic Anion Transporters (OAT1-4)

- Uptake/Influx transporters:
  - Transport organic anions against a negative membrane potential by linking to the facilitated efflux of the counter ion \( \alpha\)-ketoglutarate, whose intracellular concentration is maintained by the Na/dicarboxylate co-transporter acting in concert with the Na/K ATPase.

- Expresses in liver and kidney proximal tubules (and other tissues)

- Substrate specificity: Broad range of low Mr substrates;
  - Endogenous: cGMP, bile salts, citric acid cycle intermediates, hormones
  - Drugs: Methotrexate (anti-cancer), NSAIDs, Captopril (ACE Inhib), furosemide (diuretic), antibiotics, ganciclovir & cidofovir (anti-viral).

Clinical significance of OAT Transporters: Drug Interactions I

Methotrexate and NSAIDs drug interaction:

- Methotrexate – narrow therapeutic index
  - transported into renal tubules by OAT1
  - transported from blood to kidney via OAT1

- NSAIDs – competitive inhibitors of OAT1 transporter activity

Methotrexate

\[ \text{OAT1-dependent uptake from blood} \]

\[ \text{NSAIDs} \]

Methotrexate elimination

Clinical significance of OAT Transporters: Drug Interactions II

Using Probenecid to prevent Cidofovir-induced nephrotoxicity

- Probenecid – a potent inhibitor of OAT1

- Cidofovir: anti-viral used to treat CMV retinitis
  - transported into renal tubules by OAT1
  - always co-administered with probenecid

Cidofovir

\[ \text{OAT1 dependent uptake from blood} \]

\[ \text{Probenecid} \]

Cidofovir elimination (glomerular filtration)
B. OATP: Organic Anion Transporting Polypeptides

- Uptake/Influx transporters: Electronneutral exchangers
  - Transports substrates in exchange for $\text{HCO}_3^-$

- Broadly expressed in many tissues including gut, liver and kidney proximal tubules

- Substrate specificity: Broad range of substrates; Amphipathic Anions, $M_r > 350$ Da
  - Endogenous: Bile acids, steroids, thyroid hormones
  - Drugs: Statins, Antibiotics, Anti-cancer drugs, Anti-diabetes drugs
  - Inhibitors: Cyclosporin, Macrolide antibiotics, Flavonoids

Clinical significance of OATP transporters

- OATP1B1:
  - Important in response to STATIN drugs (anti-cholesterol)
  - Responsible for STATIN's principle effects in the liver (First Pass Effect)
  - Multiple SNP in OATP1B1 influence STATIN efficacy & systemic exposure
  - OATP1B1*15 has transporter activity
  - Cyclosporin is potent inhibitor of OATP1B1 and blocks STATIN uptake

- OATP1B1*5 and OATP1B1*15 have transporter activity
  - STATIN uptake
  - STATIN efficacy
  - Systemic exposure
  - STATIN toxicity

C. OCT: Organic Cation Transporters

- Uptake/Influx transporters: OCT1 & OCT2
  - Mediate simple passive facilitated diffusion of substrates
  - (Na+/H+ independent)

- Expressed in Gut, Kidney and Liver and other tissues

- Substrate specificity: Small positively charged compounds
  - Endogenous: monoamine neurotransmitters, creatine, catecholamines
  - Drugs: Cisplatin (chemotherapy), Metformin (anti-diabetes), Imatinib (anti-cancer), Cimetidine (H2 receptor antagonist), Procainamide (antiarrhythmic with narrow therapeutic window)
D. MATE: Multidrug and Toxin Extrusion transporters

- Efflux transporters
  - Transport organic cations
    - Secondary active transport driven by pH-antiparallel
    - Plays a major role in secretion of cationic drugs
    - Overlapping substrate specificity with OCTs
    - Primarily responsible for secretion of OCT transported substrates
  - Expressed in liver and kidney luminal brush border surfaces
  - Responsible for: renal tubular secretion of cationic drugs into urine
  - Hepatic elimination of cationic drugs into bile

Clinical significance of OCT/MATE transporters

6. Transporter polymorphisms
   - Influence the PK of multiple Organic Cation drugs (especially metformin)
   - Metformin is a common oral anti-diabetic drug that acts in the liver and is eliminated unchanged by renal tubular excretion
   - OCT/MATE transporters
     -IMPACTS renal elimination
     -POTENTIATES drug availability

B. Drug Interactions
   - Most drug interactions mediated by OCT/MATE are caused by CIMETIDINE
     - An histamine H2 receptor antagonist used in the treatment of acid peptic disorders
     - Excessively eliminated via the kidney
     - Inhibits competitive victimization of OCT transporters and prevents renal elimination of other OCT-dependent drugs
     - e.g. PROCAINAMIDE
       - Blocks PROCAINAMIDE renal elimination
       - Potentiation concentration of PROCAINAMIDE (narrow therapeutic window)

C. Prevention of Cisplatin-induced nephrotoxicity
   - Cisplatin is a chemotherapeutic agent used in the treatment of certain cancers
     - Primarily eliminated via renal tubular excretion
     - Use is limited by nephrotoxicity
     - Coinadministration of CIMETIDINE blocks Cisplatin uptake into the kidney and prevents Cisplatin-induced nephrotoxicity

ABC: ATP-binding Cassette family of Efflux transporters

A. Pglyprotein (Pgp)/Multidrug Resistant protein 1 (MDR1)
B. Breast Cancer Resistant Protein (BCRP)
C. Multidrug resistant protein (MRP)

- Active transport Efflux transporters
  - Use hydrolysis of ATP to generate the energy needed to move substrates across membranes against their concentration gradient
  - Present on the apical/luminal brush border membranes of gut, liver, and kidney epithelia
    - Involved in the active secretion of drugs across epithelial surfaces into the gut lumen, urine, and bile
    - Major role in systemic drug elimination
  - Expressed on endothelial cells of the Blood Brain Barrier (BBB)
    - Prevent access of xenobiotic compounds (drugs) to the CNS
  - Upregulated in certain cancer cells
    - Implicated in resistance of cancer cells to chemotherapeutic drugs
A. P-glycoprotein (P-gp) / MultiDrug Resistant protein-1 (MDR1)

- **Blood**
- **Gut Lumen**
- **Liver**
- **Brain**

Drug pumps drug out of the cell across the membrane and into lumen

**Drug elimination**

**Substrate Specificity:**
- Broad substrate specificity
- Typically bulky hydrophobic structures with neutral/positive charge
- Specificity overlaps with CYP34A

**Drugs:**
- Statins (narrow therapeutic range), CoX-2 inhibitors; anti-cancer drugs (e.g. paclitaxel; cytarabine)
- HIV protease inhibitors; anti-cancer drugs (e.g. etoposide; oxanthracyclines)
- Transcriptional regulators; rapamycin
- Gastrointestinal: prokinetic, antisecretory;

**Inhibitors:**
- Cyclosporin; verapamil; phosphatidylcholine; rifampin

**Inducers:** Rifampin; St. John’s wort - both induce expression of P-gp resulting in drug efflux & drug plasma concentration

**Similar to CYP34A**

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B. Breast Cancer Resistant Protein (BCRP)

- **Expression:** gut enterocytes & liver; blood brain barrier; mammary epithelium
- **Substrates:** Neutral/negatively charged compounds
- **Endogenous:** Riboflavin (Vit B12); BCRP responsible for concentrating in breast milk

**Drugs:**
- Statins; antibiotics; etoposide; imatinib & gefitinib (anti-cancer drugs)

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C. Multidrug resistant proteins (MRP)

- **Expression:** Broadly expressed in many tissues
- **Substrates:** Amphipathic molecules with at least one charged group
- **Endogenous substrates:** Glutathione, glucuronide, and sulfate conjugates
- **Drugs:** Anthracyclines, vinca alkaloids, etoposide, vincristine, methotrexate, aminoglycosides

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**The Blood Brain Barrier**

- Main function is to protect the brain from xenobiotics and toxins
- Formed by specialized brain endothelial cells - tight junctions prevent ions and large molecules passing between endothelial cells
- P-gp/MRP/BCRP ABC family efflux pumps form a barrier to a large range of drugs and other compounds
- Transport these compounds back into the blood and thereby prevent their entry into the CNS
- Excludes most drugs other than those small (< 400Da) and lipophilic—consequently only ~1% of all drugs gain access and are active in the CNS

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**Barrier Function:** Prevents drugs from entering the CNS
Clinical significance of the P-glycoprotein/MDR1 transporter I

A. Effects of P-gp/MDR1 inhibitors on drug pharmacokinetics
- P-gp/MDR1 inhibitors (e.g., cyclosporine) inhibit P-gp/MDR1 efflux activity
- In the gut, kidney, and liver this leads to decreased drug elimination of P-gp/MDR1 substrates such as DIGOXIN (narrow therapeutic window)
- Leads to increased systemic drug bioavailability and increased risk of drug toxicity

B. Inducers of P-gp e.g. RIFAMPICIN and St. John's wort (popular herbal medication)
- Induce increased expression of P-gp (and other ABC Cassette transporters)
- \(\rightarrow\) Drug efflux in gut/liver/ kidney \(\rightarrow\) Plasma concentration \(\rightarrow\) Drug efficacy

Clinical significance of the P-glycoprotein/MDR1 transporter II

C. Effect of P-gp/MDR1 inhibitors on the Blood Brain Barrier
P-gp inhibitors can decrease the efficacy of the BBB

EXAMPLE:
- LOPERAMIDE is an opioid receptor agonist used in the treatment of diarrhea
- Potent substrate for P-gp therefore \(\text{does not cross the BBB}\)
- Co-administration with P-gp inhibitors (e.g. CYCLOSPORIN) inhibits P-gp in BBB
  This allows Loperamide to cross BBB and enter the CNS
  where it can cause respiratory depression (adverse effect)

D. Effects of increased expression of P-gp/MDR1 in tumor cells
- Tumor cells often "upregulate" expression of P-gp/MDR1
- Increased expression of P-gp/MDR1 in tumor cells promotes efflux of anti-cancer drugs
- Increased expression of P-gp/MDR1 in tumor cells is associated with more aggressive phenotype, poorer prognosis and decreased sensitivity to chemotherapeutic drugs

Overview of the role of drug transporters in drug disposition
Summary: Clinical significance of drug transporters

**Pharmacokinetics**

- Drug #2 binds transporter and prevents transport of Drug #1
- Drug #1 is specifically taken up into tissue via a specific drug transporter

**Toxicity**
- Plasma concentration of Drug #1 reduced
- Efficacy of Drug #1 due to decreased uptake

**Drug Interactions**

<table>
<thead>
<tr>
<th>Type</th>
<th>Substrate</th>
<th>Prototypical Drug</th>
<th>Inhibitors</th>
<th>Clinical significance/Drug Interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>OAT</td>
<td>Uptake</td>
<td>Methotrexate</td>
<td>NSAIDs</td>
<td>Block methotrexate elimination</td>
</tr>
<tr>
<td>OATP</td>
<td>Uptake</td>
<td>Probenecid</td>
<td>Cidefovir</td>
<td>Prevents cidefovir renal uptake</td>
</tr>
<tr>
<td>OCT</td>
<td>Uptake</td>
<td>Cimetidine</td>
<td>NSAIDs</td>
<td>Block renal uptake of many drugs</td>
</tr>
<tr>
<td>MATE</td>
<td>Efflux</td>
<td>Cyclosporin</td>
<td>Probenecid</td>
<td>Block renal uptake of digoxin</td>
</tr>
<tr>
<td>P-gp</td>
<td>Efflux</td>
<td>Rifampicin</td>
<td>St. John's Wort</td>
<td>Induce P-gp expression, increase drug efflux</td>
</tr>
</tbody>
</table>

**Main role:** Renal tubular secretion of OCT substrates

- Cimetidine blocks OCT-mediated renal uptake of many drugs, thereby blocking their elimination, leading to higher plasma concentration.
- Cimetidine blocks renal uptake of cisplatin, preventing cisplatin-induced nephrotoxicity.
- Cyclosporin inhibits P-gp-mediated elimination of digoxin, increasing systemic availability.
- Inhibitors enhance CNS accessibility by overcoming BBB.

**SUMMARY**

- OATs: Cation transporters
  - Uptake linked to a KG antiport
  - Bicarbonate exchanger
  - Passive facilitated diffusion

- OCTs: Organic anion transporters
  - Broad range
  - Low Mr (Da)
  - Amphipathic anions Mr>350 Da
  - Small cations

- MATEs: Multifunctional amino acid transporters
  - Broad specificity
  - Bulky hydrophobic Neutral/+ve charge

- P-gps: ATP-dependent efflux pumps
  - ATP-dependent
  - Membrane susceptibility
  - High expression in multidrug-resistant cancer cells
  - Low in normal cells

- Inhibitors of P-gps can enhance CNS accessibility.