Learning objectives, Lec 2

Drug Transport and Intro to Drug – Target Interactions

- Major classes of drug transporters for influx / efflux ABC (ATP binding cassette) and SLC (Solute carrier)
- Intestine / Liver structural organization - P-gp transporters
- First Pass metabolism of drugs
- Enterohepatic circulation of drugs
- Example with statin drugs – role of chemical properties on first pass metabolism, systemic penetration, efficacy and adverse effects of drugs
- Example: MDR1 and digoxin
- Specialized barriers affecting drug disposition – the Blood Brain Barrier
- Challenges in drug development of CNS drugs – delivery /CNS penetration (Assigned Reichel paper).

ABC Drug Transporters

MDR1 (P-gp) and related MDR efflux pumps

- Efflux pumps (outward active transport)
  - remove xenobiotics from cells or tissues for elimination from body
  - active transport of xenobiotics using ATP as energy source for pump (fast, effective)

- MDR1 (P-glycoprotein; P-gp, ABCB1)
  - is an active transport pump in many cells
    - liver hepatocytes
    - intestinal enterocytes (gut wall)
    - brain capillary endothelium (BBB)
  - Multiple Drug Resistance in cancer cells
  - protein pump with high affinity for lipophiles
  - embedded in cell membrane
  - pumps out wide range of lipophilic drugs
  - inducible -- intracellular defense system
MDR1 (ABCB1) is the most important drug transporter yet identified

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<tr>
<th>Trans.</th>
<th>Tissue</th>
<th>Role</th>
<th>Substrates</th>
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<tbody>
<tr>
<td>MDR1</td>
<td>Liver</td>
<td>Detox of</td>
<td>Char: Neutral / Cationic / Bulky struct</td>
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<tr>
<td>(ABCB1)</td>
<td>Kidney</td>
<td>Xenobiotics?</td>
<td>Anticancer drugs: etoposide, doxorubicin, vincristine</td>
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<tr>
<td></td>
<td>Intestine</td>
<td></td>
<td>Ca2+ chan block: diltiazem, verapamil</td>
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<td></td>
<td>BBB</td>
<td></td>
<td>HIV protease inhib: indinavir, ritonavir</td>
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<tr>
<td></td>
<td>BTB</td>
<td></td>
<td>Antibiotics: Erythromycin</td>
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<tr>
<td></td>
<td>BPB</td>
<td></td>
<td>Hormones: testosterone, progesterone</td>
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<td></td>
<td></td>
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<td>Immunosuppress: cyclosporine, tacrolimus,</td>
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<td></td>
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<td>Others: digoxin, quinidine</td>
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- MDR1, MPR2, BCRP, BSEP, MDR3 in bile canaliclar membrane of hepatocytes mediate efflux (excretion) of drugs and their metabolites against steep concentration gradient from liver to bile.

- Specific tissues express inducible MDR transporters

Background relevant to drug efflux...
Intestine / Liver: Structural organization

Substances absorbed from the GI tract first travel to liver via portal blood
Drug metabolism and MDR (PgP) pump in intestinal enterocytes

- Intestinal cells actively transport drugs back into GI lumen
- Some portion of parent drug/metabolite enters portal blood

Liver Structure: Hepatic Lobule

- Liver is divided histologically into lobules
- The center of the lobule is the central vein
- At the periphery of the lobule are portal triads
Liver lobule structure facilitates drug removal via MDRs before reaching systemic circulation

Hepatic Lobules and Zones of Function

Functionally, the liver lobules divided into three zones, based upon oxygen supply

- **Zone 1** encircles the portal tracts where the oxygenated blood from hepatic arteries enters and mixes with portal blood
- **Zone 3** is located around central veins where blood exits; oxygenation is low
“First-pass metabolism” and MDR (P-gp) efflux of drugs in small intestine / liver

Oral Drug
A) 100% absorbed
B) 30% reaches portal blood circ.
C) 15% reaches systemic circ.

Due to efficient efflux by MDRs

Fig. 1: Sequential first-pass elimination of a theoretical drug through metabolism by CYP3A4 and/or transport by P-glycoprotein (P-gp) in enterocytes of the small intestine and then hepatocytes of the liver. The percentage of the initial drug dose that is available before and after passage through the gut wall and liver is presented. Although the drug is 100% absorbed from the gastrointestinal tract, its bioavailability is only 15% after oral administration.

Drugs subject to significant ‘first pass effects’:
morphine, propranolol, buprenorphine, diazepam, midazolam, demerol, cimetidine

Enterohepatic Circulation
• Prolongs half-life of many drugs

- Liver excretes lipophiles into bile via active transport pump
- Lipophiles excreted into small intestine w/bile
  - some fraction of the lipophile is excreted in feces
- Remaining fraction is reabsorbed from GIT into portal blood via passive diffusion
- Enterohepatic cycle prolongs half-life of many lipophiles

Systemic circulation
- Bile
- Drug is metabolized
- Carrier-mediated transport system
- Renal excretion
- Drug is absorbed, metabolized, excreted
**URINARY EXCRETION**

- Most compounds eliminated by kidneys
- Polar and ionic compounds
- Small MW (<600 Da)

**Ex: Statins - HMG-CoA Reductase Inhibitors**

- Statins = cholesterol-lowering drugs via reversible inhibition of HMG-CoA reductase (catalyzes rate-limiting step in cholesterol biosynthesis in liver)
- Exposure of extrahepatic cells causes adverse effects (rhabdomyolysis, myopathy)

**Simvastatin**

**Parvastatin**

**Atorvastatin (Lipitor)**
Ex: HMG-CoA Reductase Inhibitors

- Some statins (pravastatin, fluvastatin, etc.) given in a biologically active open-acid form
- Others (simvastatin and lovastatin) given as prodrugs w/lactone rings
  - have somewhat greater systemic penetration.

- Open-acid statins are more hydrophilic → lower membrane permeability
- Substrates of uptake transporters (OATP1B1) and efflux transporters (MRP2) → efficient hepatic uptake and elimination (vectoral transport) → enterohepatic circulation
- Maximizes action in hepatocytes (site of cholesterol synthesis) and minimizes ‘escape’ into systemic circulation (and not target adverse effects)

MDR1 substrates: MDR1 and Digoxin

1. Diazepam: Positive allosteric modulator of GABA$_A$
2. Dimercaprol (BAL): Metal chelator
3. Theophylline: Competitive nonselective phosphodiesterase inhibitor → inc. cAMP, PKA, inhib. TNF-a and inflammation.
4. Warfarin: Vit K inhib, anti-coagulant
5. Digoxin:

- Dixogin: purified cardiac glycoside (foxglove)
- Atrial fibrillation, atrial flutter, etc.
  - Increases myocardial contractility, but also increases contraction duration → decreased heart rate, increased stroke vol, increased BP and tissue perfusion = increased myocardial efficiency.
  - No longer widely used (ex patients unresponsive to ACE inhib, etc.) because was not effective in reducing morbidity or mortality in CHF cases
- Digoxin interaction with Verapamil (L-type Ca channel blocker to treat hypertension).
  - Verapamil increases plasma digoxin levels by displacing tissue binding sites and depressing renal digoxin clearance
  - Verapamil is MDR1 inhibitor
    - TI for diazepam ~ 100
    - TI for digoxin ~ 2 – 3
Challenges in Drug Delivery to Target Sites...

**REVIEW**

Addressing Central Nervous System (CNS) Penetration in Drug Discovery: Basics and Implications of the Evolving New Concept

by Andreas Reichel

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The lifecycle of a neurotransmitter

Presynaptic terminal
Vesicle just released transmitter
Synaptic cleft
Postsynaptic terminal

**NT Lifecycle**
1. Synthesis
2. Storage
3. Release
4. Binding
5. Destruction

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Serotonin Synaptic Function

- Serotonin is most commonly inhibitory.
- PS cell's response is influenced by the amount of serotonin in the cleft and by the types of receptors; serotonin receptors come in at least 13 "sub-types".
- Serotonin levels in synapses are reduced by: 1) autoreceptors (orange) \(\rightarrow\) signal to reduce serotonin production, and 2) reuptake transporters (SERT, SLC6A4 yellow), which absorb the neurotransmitter.
- Several antidepressants (fluoxetine – Prozac, and paroxetine – Paxil, and MDMA, increase synaptic serotonin by inhibiting its reuptake.

SERT is the primary target of Ecstasy - MDMA (methylene-dioxymethamphetamine)

Blood Supply to the Brain

Animation of CNS circulation:
http://www.youtube.com/watch?v=jCf273U0ktc&feature=player_embedded
Brain capillary endothelial cells express diverse array of transmembrane transporters
Lipid solubility and the brain uptake of compounds

- Higher the Kow, the greater the brain uptake
- Specific carriers can facilitate brain uptake (glucose, lucine, dopa)
- Binding to plasma proteins can reduce uptake (phenytoin)

Problem: Achieving a safe and efficacious concentration profile in the brain remains one of the big challenges in central nervous system (CNS) drug discovery and development

- Currently, PK support of CNS drug discovery heavily relies on improving the blood–brain barrier (BBB) permeability in vitro and/or the brain/plasma ratio (Kp) in vivo.
  - Neither parameter is reliably linked to pharmacodynamic (PD) and efficacy outcomes.
- While increasing BBB permeability may shorten the onset of drug action, an increase in the total amount in brain may not necessarily increase the relevant drug concentration at the pharmacological target.
- The traditional Kp B/P ratio is based on a crude homogenization of brain tissue, it ignores the compartmentalization of the brain and an increase favors non-specific binding to brain lipids rather than free drug levels.
- The complex nature of the brain requires different compartments to be considered when trying to understand and improve new compounds, several complementary parameters need to be measured in vitro and in vivo, and integrated into a coherent model of brain penetration and distribution.
- The new paradigm thus concentrates on finding drug candidates with the right balance between free fraction in plasma and brain, and between rate and extent of CNS penetration. CNS Diseases are traditionally defined by clinical symptoms rather than pharmacological mechanisms, hence there often is a large fraction of non-responders to mechanistic drug candidates in the patient population in the clinic, masking the effect in a potentially responding sub-population.
- Consequently, scientists must address ADME processes from early on in the drug discovery process in order to optimize and balance the various compound properties so to increase the chances of success in the clinic.
Ex: Morphine-6-O-glucuronide (M6G)

1. M6G has a much lower extent and slower rate of brain penetration than morphine
2. But M6G shows a similarly high central analgesic efficacy

- This is unexplained by the classic concept of CNS penetration, e.g.,
  - M6G has a poor brain/plasma ratio of 0.069 Vs 0.54 for morphine
  - M6G has a lower rate of BBB permeation, expressed as permeability surface area (PS) product of 0.11 Vs 3.5 mL/min/g brain for morphine

- Both have similar affinity for the opioid receptor.
- M6G should show no in vivo efficacy at all, ... BUT IT IS EQUALLY EFFICACIOUS

<table>
<thead>
<tr>
<th></th>
<th>Morphine</th>
<th>Morphine-6-O-glucuronide</th>
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<tbody>
<tr>
<td>PS [μL/min/g brain]</td>
<td>3.5</td>
<td>0.11</td>
</tr>
<tr>
<td>Kp</td>
<td>0.54</td>
<td>0.07</td>
</tr>
<tr>
<td>Kp</td>
<td>0.65</td>
<td>0.08</td>
</tr>
<tr>
<td>Kp</td>
<td>0.29</td>
<td>0.29</td>
</tr>
<tr>
<td>Vd [μL/g brain]</td>
<td>2.1</td>
<td>0.11</td>
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</table>

Although total brain levels of M6G are much lower than those of morphine, the exposure at site of the target receptor, i.e., the levels within the ISF, is even higher for M6G (four-times those of morphine)

Recommendations from Reichel...

Must:
1) Brain is complex – use integrated model of drug penetration and distribution
2) Emphasize Drug leads that optimize:
   - Free plasma and free brain fractions of drug
   - Rate and extent of CNS penetration
3) Address ADME processes from the start
Kin = influx clearance into brain [ml/min/g brain]
PS = permeability surface area product [ml/min/g brain]
Qbr = cerebral blood flow [ml/min/g brain]
Kp = total brain to total plasma concentration ratio
Kp,uu = unbound brain to unbound plasma concentration ratio
fu,brain = fraction unbound in brain
fu,plasma = fraction unbound in plasma

very poor correlation between fu,plasma and fu,brain

Likely due to very different lipid and protein, composition of plasma and brain,
- plasma has 2x as much (protein vs brain
- while brain has 20-fold more lipids
New concept: Clear differentiation between/integration of:

- rate (of BBB permeation),
- extent (of brain penetration),
- distribution (within the CNS).

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<thead>
<tr>
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<th>Classic PK</th>
<th>CNS PK</th>
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<tbody>
<tr>
<td>1) Rate</td>
<td>‘Elimination’ clearance</td>
<td>‘Uptake’ clearance</td>
</tr>
<tr>
<td></td>
<td>$CL = \frac{Dose}{AUC_{total}}$</td>
<td>$K_u = \frac{AUC_{brain}}{AUC_{total}}$</td>
</tr>
<tr>
<td>2) Extent</td>
<td>Extent of oral bioavailability</td>
<td>Extent of brain uptake</td>
</tr>
<tr>
<td></td>
<td>$F = \frac{AUC_{brain}}{AUC_{total}}$</td>
<td>$K_p = \frac{AUC_{brain}}{AUC_{total}}$</td>
</tr>
<tr>
<td>3) Distribution</td>
<td>Concept of total vs. unbound concentrations</td>
<td>$V_{e,q,brain} = V_{e,q,brain}$</td>
</tr>
<tr>
<td>4) Half-life</td>
<td>Half-life of elimination</td>
<td>Half-life to equilibrium</td>
</tr>
<tr>
<td></td>
<td>$T_{\frac{1}{2}, e,q} = \frac{\ln 2 \times V_e}{CL}$</td>
<td>$T_{\frac{1}{2}, e,q} = \frac{\ln 2 \times V_{e,q,brain}}{PS \times f_{brain}}$</td>
</tr>
</tbody>
</table>

**Types of studies to obtain concentrations in the compartments:**

1. In vivo PK
2. In vitro plasma protein binding
3. In vitro binding to brain homogenate

**Fig. 4.** Principal pharmacokinetic compartments of the CNS and the relation between bound and unbound concentrations in the compartments. The dark boxes illustrate parameters which can be measured in vitro and in vivo, and their relation to the concentration in these PK compartments. The three boxes at the bottom summarize the methods by which the parameters shown can be obtained: 1) in vivo determination of the total brain to total plasma ratio $K_p$, the total amount of drug in brain $A_{brain}$, and total plasma concentrations $C_{e,plasma}$; 2) and 3) equilibrium dialysis of blood plasma and brain homogenate giving the fraction unbound in plasma and brain, from which the unbound concentrations in plasma $C_{e,plasma}$ and brain $C_{e,brain}$ from the in vivo study (1) can be derived.