Permeability-glycoprotein (P-gp): Efflux Transporter

1. Transports drugs out of cells in many locations – e.g., placenta, brain and testes
2. Specific component of blood-brain barrier
3. Loperamide (Imodium®) is a potent opiate that acts on gut to slow motility – but no actions in brain.

| A | Absorption | YES |
| D | Distribution | YES |
| M | Metabolism | YES |
| E | Excretion | YES |

Delivering drugs to the brain is a huge challenge

The Blood-Brain Barrier: Bottleneck in Brain Drug Development

Whole-body autoradiogram of radiolabeled histamine

*Pardridge, 2012, JCBFM, 32, 1959
*Pardridge, 2005, NeuroRX
Delivering drugs to the brain is a huge challenge

BUI = brain uptake index

Many factors affect brain penetration - logP

Diffusion vs. transport

Drug Discovery Today

The promiscuous binding of pharmaceutical drugs and their transporter-mediated uptake into cells: what we need to know and how we can do so

Evidence-based approach to assess passive diffusion and carrier-mediated drug transport

Many factors affect brain penetration - logP

Active uptake

Efflux transport

Fig 1: Plots CNS permeability against log P. Many solutes enter tissues by more or less linear scaling, determined by log P or DNA penetration. The solutes on the x-axis are: hydrophobic drugs (PCMU, O2, H2O, and glucose), P-glycoprotein (P-gp), ATP-binding cassette (ABC) transporters (MRP1, ABCB1), and flucloxacillin. The list indicates that there is a trend of reduced diffusion across the BBB as solute hydrophobicity increases. The increase in solute hydrophobicity is inversely proportional to the ability to cross the BBB. Levin et al. 1980. J. Med. Chem. 23: 682-684

ATP-binding cassette (ABC) transporters at the blood-brain barrier

3 most common classes:
- P-glycoprotein (P-gp/ABCB1)
- Multidrug resistance protein (Mrp1/ABCC1)
- Breast cancer resistance protein (Bcrp/ABCG2)

Limits drug delivery to brain

Increased function:
- Drug-resistant epilepsy
- HIV infection of brain
- Multidrug resistant cancer

Dysfunction:
- Alzheimer disease

Modified from Loscher et al. 2005 Nat Rev Neurosci 6: 591-602

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**Transporters at the blood-brain barrier**

<table>
<thead>
<tr>
<th>Genes</th>
<th>Protein</th>
<th>Transporter (cm/s)</th>
<th>Calculated Km (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABC1</td>
<td>-</td>
<td>2.80</td>
<td>0.63</td>
</tr>
<tr>
<td>ABC3</td>
<td>-</td>
<td>1.11</td>
<td>0.38</td>
</tr>
<tr>
<td>ABC4</td>
<td>P-gp</td>
<td>4.87</td>
<td>0.86</td>
</tr>
<tr>
<td>ABC5</td>
<td>NTp</td>
<td>0.13</td>
<td>0.04</td>
</tr>
<tr>
<td>MDR1 A</td>
<td>Pgp</td>
<td>24.3</td>
<td>5.18</td>
</tr>
<tr>
<td>MDR1 B</td>
<td>LPLP</td>
<td>1.04</td>
<td>0.01</td>
</tr>
<tr>
<td>MDR1 C</td>
<td>Pgp</td>
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<td>0.74</td>
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<tr>
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<td>RTG</td>
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<tr>
<td>MDR1 F</td>
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<tr>
<td>ENT1</td>
<td>Pgp</td>
<td>2.06</td>
<td>0.38</td>
</tr>
</tbody>
</table>

**Why study (ABC) transporters at the blood-brain barrier?**

- Understand how changes in P-gp expression impact disease states
- Can we take advantage of transport inhibitors to improve drug delivery (Snatch victory from the jaws of defeat)
- Patient imaging/diagnosis preferable to post-mortem imaging

**How to study P-gp using imaging?**
Renal Cell Carcinoma:
Tariquidar increases uptake of $^{99m}$Tc-Sestamibi in metastasis of thigh

Baseline

After Tariquidar

[S Bates and T Fojo, NCI]

$[^{11}C]$dLop: brain uptake much higher in P-gp KO than in wild type mice

[Image of MRI, WT, P-gp KO with corresponding uptake graphs]

P-gp blockade increases uptake of $[^{11}C]$dLop in monkey brain but not in pituitary.

[Image of baseline and P-gp blockade uptake in brain and pituitary]
Brain uptake of $^{11}$CdlOp increases after P-gp inhibition and is trapped in monkey brain.

$^{[11]C}dLop$: Distribution of radioactivity in healthy male

What is this?
Extended summed images (0 – 10 min) show blood pool and tissue accumulation.

Tariquidar 6 mg/kg increases $[^{11}\text{C}]\text{dLop}$ by 250%.

P-gp blockade dose-dependently increases uptake of $[^{11}\text{C}]\text{dLop}$ in human brain.
Thesis Work of Pavitra Kannan:
PhD student in NIH / Karolinska program

1. $^{[11]}$C-dLop is a selective substrate for P-gp.
2. Retention of $^{[11]}$C-dLop in brain reflects ionic trapping in acidic vesicles.

Accumulation of $^{[3]}$H-dLop is lowest in ABCB1 (P-gp) expressing cells.

Uptake of $^{[11]}$C-dLop is highest in brains of P-gp knockout mice.
Summary

1. P-glycoprotein (P-gp): efflux transporter in many organs and can block entry of drugs into brain.
2. $[^{11}C]$desmethyl-loperamide (dLop) is substrate selective for P-gp in mice, monkey, and man.
3. P-gp at blood-brain barrier acts rapidly and with high capacity to block entry of $[^{11}C]$dLop.
4. Function of P-gp in humans can be measured with $[^{11}C]$dLop at baseline and after inhibition.
5. Models are important for predicting and understanding the potential for a drug to cross the blood-brain barrier.

Acknowledgements

PET: Imaging P-gp Function
Robert Innis, MD PhD
Victor Pike, PhD: Director of "Radiopharmaceutics"
Sami Zoghbi, PhD: metabolic studies
Jeih-San Liow, PhD: animal imaging
William C. Kreisl, MD: human imaging

In vitro: P-gp Selectivity & Lysosomal Trapping
Pavitra Kannan, PhD
Michael Gottesman, MD

Brain trapping of $[^{11}C]$dLop is not due to high-affinity binding to opiate receptors.
Structure of dLop: weak base

Hypothesis: dLop ionically trapped in lysosomes

Competition with other weak bases
Lysotracker Red: fluorescent weak base that accumulates in lysosomes

Displacement of Lysotracker Red by other weak bases

Compounds that raise lysosomal pH decrease accumulation of dLop in vitro.