PET Imaging of P-gp:
an efflux transporter at blood-brain barrier

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Outline of Talk

1. Positron emission tomography (PET) has high sensitivity to measure small mass doses of radiolabeled drugs in body.

2. Loperamide (Imodium®) is a potent opiate that acts on receptors in gut, but P-gp blocks its entry into brain.

3. $^{[11]}$C]desmethyl-loperamide (dLop) is also substrate for P-gp in mice, monkey, and man.

4. dLop (weak base) is ionicly trapped in acidic vesicles.

5. $^{[11]}$C]dLop may measure function of P-gp in disease.
   * Increased function may cause drug resistance in cancer and epilepsy.
Positron Emission Tomography
### PET vs. MRI

<table>
<thead>
<tr>
<th></th>
<th>PET</th>
<th>MRI</th>
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</thead>
<tbody>
<tr>
<td><strong>Spatial Resolution</strong></td>
<td>2 – 6 mm</td>
<td>&lt;&lt; 1 mm</td>
</tr>
<tr>
<td><strong>Sensitivity</strong></td>
<td>$10^{-12}$ M</td>
<td>$10^{-4}$ M</td>
</tr>
<tr>
<td><strong>Temporal Resolution</strong></td>
<td>minutes</td>
<td>&lt;1 sec</td>
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Radionuclide ($^{11}$C): high sensitivity  
Ligand (raclopride): high selectivity  
Radioligand [$^{11}$C]raclopride: high sensitivity & selectivity
P-glycoprotein (P-gp) Efflux Transporter

1. Transports drugs out of cells in many locations – e.g., 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.
4. Over expressed in 40% of tumors resistant to chemotherapy
P-glycoprotein removes lipophilic substrates directly from the plasma membrane
[\textsuperscript{11}C]dLop: brain uptake much higher in P-gp KO than in wild type mice
P-gp blockade increases uptake of $[^{11}C]$dLop in monkey brain but not in pituitary.

Baseline

P-gp blocked with DCPQ

P-gp blockade
[11C]dLop in Monkey Brain

P-gp blockade increases brain uptake but no effect on pituitary
[\textsuperscript{11}C]dLop: Distribution of radioactivity in healthy male
Summed early images (0 – 3 min) show blood pool.
Minimal brain uptake of $^{11}$C\textit{dLop} in healthy human brain

![PET](image1)

![Fused](image2)

![MRI](image3)

![Graph](image4)
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%.
Brain uptake of $[^{11}\text{C}]d\text{Lop}$ increases dose-dependently after inhibition of P-gp.
Thesis Work of Pavitra Kannan

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.
ABC transporters at the blood-brain barrier

3 most common:
- ABCB1 (P-gp)
- ABCC1
- ABCG2

Accumulation of $[^3\text{H}]\text{dLop}$ is lowest in ABCB1 (P-gp) expressing cells.
Uptake of $[^{11}\text{C}]$dLop is highest in brains of P-gp knockout mice.
Brain uptake of $[^{11}C]$dLop increases after P-gp inhibition and is trapped.

![Graph showing the concentration of radioactivity over time after injection.](image)

**Baseline**

**DCPQ 16 mg/kg**

**Naloxone 5 mg/kg**
Structure of dLop: weak base

dLop
Hypothesis: lysosomal trapping

Weak base
pKa ~ 8.0

Cytosol

pH 7.4

Lysosome

pH 5.5

H+ H+
Competition with other weak bases

Cytosol

Lysosome

pH 7.4

pH 6.5

Accumulation of Weak base 2

[Weak base 1]

Weak base 1 (blocker)

Weak base 2 (substrate)
Displacement of Lysotracker Red by other weak bases

Baseline

Weak Base
100 µM Tamoxifen

Non-Base
10 µM Taxol

100 µM dLop
What is organ above left kidney?
Renal Cell Carcinoma: Tariquidar increases uptake of $^{99m}$Tc-Sestamibi in metastasis of thigh
Translocator protein (marker of neuroinflammatory cells) can localize epileptogenic focus.
Summary

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Self-Assessment Quiz: True or False?

• Loperamide, an antidiarrheal drug, lacks central nervous system opiate effects because P-gp (Permeability-glycoprotein) blocks its entry into brain.

• Positron emission tomography (PET) can measure the function of P-gp in vivo by using a radiolabeled P-gp substrate such as [\(^{11}\text{C}\)]loperamide.

• PET can monitor the in vivo metabolism of radioligands. By measuring P-gp function, PET can also monitor drug distribution.
Disulfiram: Decreases Skull Activity & Increases Brain Uptake

Baseline

Disulfiram

Images at 2 h in same subject. Disulfiram 500 mg PO prior night