PET Imaging of P-gp:
an efflux transporter that protects brain
but also causes drug resistance

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Outline of Talk

1. P-glycoprotein (P-gp): efflux transporter in many organs and can block entry of drugs into brain.
2. Loperamide (Imodium®) is a potent opiate that acts on receptors in gut, but P-gp blocks its entry into brain.
3. \([^{11}\text{C}]\text{desmethyl-loperamide (dLop)}\) is also substrate for P-gp in mouse, monkey, and man.
4. Blockade of P-gp increases brain uptake of \([^{11}\text{C}]\text{dLop}\).
5. \([^{11}\text{C}]\text{dLop}\) is ionically trapped in acidic lysosomes.
6. Over expression of P-gp may mediate drug resistance in cancer and epilepsy.
7. PET's amazing capabilities attract Pharma to NIH for early clinical trials
Positron Emission Tomography

PET vs. MRI

<table>
<thead>
<tr>
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<th>PET</th>
<th>MRI</th>
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<tbody>
<tr>
<td>Spatial Resolution</td>
<td>2 – 6 mm</td>
<td>&lt;&lt; 1 mm</td>
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<tr>
<td>Sensitivity</td>
<td>$10^{-12}$ M</td>
<td>$10^{-4}$ M</td>
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<tr>
<td>Temporal Resolution</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

Permeability-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 half of tumors resistant to chemotherapy
P-gp Transport in Human Body

P-glycoprotein removes lipophilic substrates directly from the plasma membrane.

[¹¹C]dLop: brain uptake much higher in P-gp KO than in wild type mice
P-gp blockade increases uptake of $[^{11}\text{C}]\text{dlOp}$ in monkey brain but not in pituitary.

Baseline

P-gp blockade

Brain uptake of $[^{11}\text{C}]\text{dlOp}$ increases after P-gp inhibition and is trapped in monkey brain.

Brain uptake depends on blood flow and single pass extraction.

$K_1 = \text{rate brain entry}$

$K_2 = \text{flow} \cdot \text{extraction}$

$K_1 = F \cdot E$

Example

Flow of drug 100 µg per min

Extraction is 2%

$K_1 = 2$ µg per min
Single Pass Extraction of $^{11}$CdLop >50%

1) Measure $K_1$ from brain and plasma data of $^{11}$C-dLop
2) Measure blood flow with $^{15}$O$_2$O
3) Calculate Extraction ($E$)

$$E = \frac{K_1}{F}$$

$K_1 > 0.25$ mL per cm$^3$ per min
$F = 0.5$ mL per cm$^3$ per min

$E > 0.5 = 50$

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

Summed early images (0 – 3 min) show blood pool.
Minimal brain uptake of $[^{11}C]dLop$ in healthy human brain

What is this?

Extended summed images (0 – 10 min) show blood pool and tissue accumulation.
Tariquidar 6 mg/kg increases $[^{11}C]$dLop by 250%.

Baseline

Tariquidar 6 mg/kg

Kreisl JNM 2010


Kreisl JNM 2010

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

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

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

Parental line
Resistant line

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

ABCB1 (P-gp) KO
ABCG2 KO
ABCC1 KO
WT
Brain trapping of $[^{11}C]$dLop is not due to high-affinity binding to opiate receptors.

Baseline

DCPQ

Naloxone

Naloxone: 5 mg/kg
DCPQ: 16 mg/kg

Structure of dLop: weak base

$pK_a = 7.3$

dLop

Hypothesis: dLop ionically trapped in lysosomes
Competition with other weak bases

Cytosol pH 7.4

Lysosome pH 6.5

Accumulation of weak base 2

Conc of weak base 1

Lysotracker Red: fluorescent weak base that accumulates in lysosomes

Displacement of Lysotracker Red by other weak bases

Baseline

Weak Base 100 µM Tamoxifen

Non-Base 10 µM Taxol

Hoechst 33342

Lysotracker Red DND-99

Merge

Kannan PNAS 2011
Compounds that raise lysosomal pH decrease accumulation of dLop in vitro.

Tariquidar has two effects: P-gp inhibition and lysosomal displacement.

Displacement of Lysotracker Red by other weak bases
Tariquidar decreases $[^{11}C]$dLop accumulation in peripheral organs of P-gp knockout mice and of humans.

**Conclusions**

1) dLop is a selective substrate for human ABCB1 (P-gp).

2) dLop, a weak base, is trapped within acidic lysosomes and can be displaced by other weak bases.

3) Some P-gp inhibitors, including tariquidar, are weak bases and "lysosomotropic."
Renal Cell Carcinoma: Tariquidar increases uptake of $^{99m}$Tc-Sestamibi in metastasis of thigh

Baseline

After Tariquidar

Overexpression of P-gp may cause drug resistance in epilepsy

- Rat model of epilepsy: P-gp overexpression
- Surgical human tissue: increased P-gp
- Rats: inflammation upregulates P-gp

Epilepsy: Inflammation & P-gp

Incidence
15 of 16 patients

Inflammation marker (TSPO)

P-gp imaging

Expect increased P-gp function at the seizure focus

Patient #1

Patient #2

Hirvonen, In press
Summary

1. P-glycoprotein (P-gp): efflux transporter in many organs and can block entry of drugs into brain.
2. [\(^{11}\text{C}\)desmethyl-loperamide (\(\text{dLop}\)) 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 [\(^{11}\text{C}\)dLop.
4. [\(^{11}\text{C}\)dLop is ionically trapped in acidic lysosomes.
5. Function of P-gp in humans can be measured with [\(^{11}\text{C}\)dLop at baseline and after inhibition.
6. Over expression of P-gp may mediate drug resistance in cancer and epilepsy.

Lazabemide blocks [\(^{11}\text{C}\)deprenyl binding to monoamine-oxidase-B (MAO-B)

Selegilene is more potent and longer acting than lazabemide

mGluR5 Receptors: Potential Clinical Applications

- **Fragile X**: mental retardation in males
  - mGluR5 knock down and antagonists block symptoms in Fragile X mice.
- **Schizophrenia**
  - mGluR5 KO mice show “schizophrenia-like” behaviors, reversed by clozapine.
  - Positive allosteric modulators (PAMs) reverse NMDA-induced effects.
PET can attract Pharma to NIH for early clinical trials

- **mGluR5 Receptor**
  - Negative allosteric modulator (NAM): Fragile X and autism via Seaside Therapeutics
  - Positive allosteric modulator (PAM): schizophrenia

- **Nociceptin Orphanin Peptide (NOP) Receptor**
  - Agonist: anxiety via company #1
  - Antagonist: anxiety via company #2

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**Receptor density (B_max) of mGluR5 is significantly higher in Fragile-X than in control subjects.**

![Graph showing receptor density comparison between Healthy and Fragile-X subjects.](image)

- % Change in B_max = + 19%
- Two-tailed p = 0.029

Lohith, In preparation

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**Global Conclusions and Future Directions**

- PET is an effective tool for studying pathophysiology and facilitating therapeutic drug development.

- PET has and is being used to attract Pharma to NIH for early clinical trials.
**ACKNOWLEDGEMENTS**

**PET: Imaging P-gp Function**
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 candidate, Karolinska / NIH
Matt Hall, PhD, senior fellow
Michael Gottesman, MD

**Epilepsy**
Jussi Hirvonen, MD, PhD
William Theodore, MD

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**Human diseases associated with an ABC Transporter**

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<tr>
<th>Disease</th>
<th>Transporter</th>
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<tbody>
<tr>
<td>Cancer</td>
<td>ABCB1 (MDR1), ABCC1 (MRP1), ABCG2 (MRX)</td>
</tr>
<tr>
<td>Cystic fibrosis</td>
<td>ABCC7 (CFTR)</td>
</tr>
<tr>
<td>Stargardt disease &amp; AMD</td>
<td>ABCA4 (ABCR)</td>
</tr>
<tr>
<td>Tangier Disease and Familial HDL deficiency</td>
<td>ABCA1 (ABci)</td>
</tr>
<tr>
<td>Progressive familial intrahepatic cholestasis</td>
<td>ABCB11 (SPGP), ABCB4 (MDR2)</td>
</tr>
<tr>
<td>Dubin-Johnson syndrome</td>
<td>ABCB2 (MRP2)</td>
</tr>
<tr>
<td>Pseudoxanthoma elasticum</td>
<td>ABCC6 (MRP6)</td>
</tr>
<tr>
<td>Persistent hypoglycemia of infancy</td>
<td>ABCC8 (SLC1), ABCC9 (SLC2)</td>
</tr>
<tr>
<td>Sideroblastic anemia and ataxia</td>
<td>ABCB7 (ABC1)</td>
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<tr>
<td>Adrenoleukodystrophy</td>
<td>ABCG1 (ALD)</td>
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<tr>
<td>Sjogren's syndrome</td>
<td>ABCG5, ABCG8</td>
</tr>
<tr>
<td>Immune deficiency</td>
<td>ABCB2 (Tap1), ABCB1 (Tap2)</td>
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