PET Imaging of P-gp

efflux transporter at blood-brain barrier

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Graphic

Outline of Talk

1. **P-gp (permeability glycoprotein) is an ATP-binding cassette (ABC) transporter. Located throughout body, P-gp affects distribution and excretion of its substrates.**
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. **When P-gp is fully blocked, [^{11}C]dLop has very high brain uptake (>50% single pass extraction) and is 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

Title slide from a slide show by Dr. Simon R. Chory, Ph.D.

Positron Emission Tomography
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PET vs. MRI

	PET	MRI
Spatial Resolution	2 – 6 mm	<< 1 mm
Sensitivity	10^{-12} M	10^{-4} M
Temporal Resolution	minutes	<1 sec

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

Graphic

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

MRI, WT, and P-gp KO images showing this process.

Chart showing Conc Activity (% SUV) from 0 to 100 over Time (min) from 0 to 100 minutes. P-gp KO is at a much higher level (around 80% down to approximately 70% SUV at the end of the process) compared to WT (from around 40 % SUV down to approximately 12% SUV at the end of the process).

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

PET monkey brain images showing the pituitary gland at baseline and at P-gp blockade (P-gp blocked with DCPQ)

[^{11}C]dLop in Monkey Brain

P-gp blockade increases brain uptake but no effect on pituitary

Two plots are shown. One is of the baseline showing radioactivity concentration (%SUV) from 0 to 400 over time after injection (min) from 0 to 125. The other plot shows DCPQ (8 mg/kg) radioactivity concentration (%SUV) from 0 to 500 over time after injection (min) from 0 to 125

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

Radiographs showing distribution of radioactivity in the brain, lung, kidney, thyroid, spleen, liver, and urinary bladder over time (min) from 3 to 100.

Summed early images (0 – 3 min) show blood pool.

Radiograph showing this

Minimal brain uptake of [^{11}C]dLop in healthy human brain

PET, Fused, and MRI images illustrating this uptake.

Graph showing conc radioactivity (%SUV) from 0 to 50 over time after injection (min) from 0 to 100 for whole brain and whole brain-vascular corrected.

What is this?

Comparison of PET, Fused, and MRI images

PET FUSED MRI

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

Tariquidar 6 mg/kg increases [^{11}C]dLop by 250%, but “therapeutic” dose (2 mg/kg) by only 20%.

Comparison of PET and MRI images at baseline and with Tariquidar 6 mg/kg (%SUV) from 0 to 400.

Brain uptake of [^{11}C]dLop increases in a dose-dependent manner after inhibition of P-gp

Plot showing brain uptake (%SUV · min) from 0 to 1500 over Tariquidar dose (mg · kg^{-1}) from 0 to 7. Brain update with 0 to 2 Tariquidar dose (mg · kg^{1-}) is approximately 250 %SUV · min and then it rapidly rises to about 1000 %SUV · min at approximately 6 Tariquidar dose (mg · kg^{1-}).

Thesis Work of Pavitra Kannan

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

ABC transporters at the blood-brain barrier

Graphic

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

Loscher *et al.* 2005. *Nature Review Neuroscience.* Drug resistance in brain diseases

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

Bar chart showing accumulation [^{3}H]dLop (fmol / 10^6 cells) from 0 to 250 in a parental line and a resistant line of ABCB1, ABCG2, and ABCC1.

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

Plot of Conc radioactivity (%SUV) from 0 to 35 over time after injection (min) from 0 to 60

1. Brain uptake of [^{11}C]dLop increases after P-gp inhibition and is trapped

Plot of Pituitary and Whole brain by Conc radioactivity (%SUV) from 0 to 800 over time after injection (min) from 0 to 90

Structure of dLop: weak base

Hypothesis: lysosomal trapping

Graphic and chemical structure showing the weak base pKa ~ 8.0.

Competition with other weak bases

Graphic depicting weak base 1 (displacer) and weak base 2 (substrate)

Displacement of Lysotracker Red by other weak bases

PET images at Baseline, Weak Base 100 μM Tamoxifen, Non-Base 10 μM Taxol, 100 μM dLop, and 500 nM Tariquidar.

What is organ above left kidney?

Renal Cell Carcinoma: Tariquidar increases uptake of ^{99m}Tc-Sestamibi in metastasis of thigh

PET images at Baseline at 1 hour, 2 hours, and 3 hours and after Tariquidar at 1 hour, 2 hours and 3 hours.

Translocatorprotein (marker of neuroinflammatory cells) can localize epileptogenic focus

Summary

- **P-gp (permeability glycoprotein) is an ATP-binding cassette (ABC) transporter. Located throughout body, P-gp affects distribution and excretion of its substrates.**
- **Loperamide (Imodium®) is a potent opiate that acts on receptors in gut, but P-gp blocks its entry into brain.**
- **[^{11}C]desmethyl-loperamide (dLop) is also substrate for P-gp in mice, monkey, and man.**
- **When P-gp is fully blocked, [^{11}C]dLop has very high brain uptake (>50% single pass extraction) and is trapped in acidic vesicles.**
- **[^{11}C]dLop may measure function of P-gp in disease.**
  - *** Increased function may cause drug resistance in cancer and epilepsy.**

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}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

PET images at Baseline and Disulfiram. Images at 2 h in same subject. Disulfiram 500 mg PO prior night.

Baseline Disulfiram

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