

PET Imaging of P-gp

- efflux transporter at blood-brain barrier and
- a mechanism for multidrug resistance in cancer

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Outline of Talk

- **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.**
- **[¹¹C]desmethyl-loperamide (dLop) is also substrate for P-gp in mice, monkey, and man.**
- **When P-gp is fully blocked, [¹¹C]dLop has very high brain uptake (>50% single pass extraction) and is trapped in acidic vesicles.**
- **[¹¹C]dLop may measure function of P-gp in disease.**
 - * **Increased function may cause drug resistance in cancer and epilepsy.**

Imaging of neuroreceptors by PET

Positron Emission Tomography

PET vs. MRI

	PET	MRI
Spatial Resolution	2 – 6 mm	$\ll 1$ 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

What are ABC transporters?

- ABC = ATP-Binding Cassette
- Hydrolyze ATP to efflux drugs (substrates)
- Protect organs from entry of drugs/toxins

P-glycoprotein (P-gp) Efflux Transporter

- Transports drugs out of cells in many locations – e.g., brain and testes
- Specific component of blood-brain barrier
- Loperamide (Imodium®) is a potent opiate that acts on gut to slow motility – but no actions in brain.
- Over expressed in 40% 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 [¹¹C]dLop in monkey brain but not in pituitary.

[¹¹C]dLop in Monkey Brain

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

P-gp Inhibitor Increases Uptake of [11C]dLop into Four Regions of Monkey Brain.

Linear increase.

Maximal effect is >16 mg/kg i.v.

**[¹¹C]dLop in Monkey Brain:
Radioligand does not bind to opiate receptors**

DCPQ 16 mg/kg, Naloxone 5 mg/kg (30 min after injection)

Is P-gp function uniformly distributed
in brain?

FC=Frontal Cortex, AC=Anterior Cingulate Gyrus, TE=Temporal Cortex , PA=Parietal
Cortex,

HP=Hippocampus, OC= Occipital Cortex, PU=Putamen, CE=Cerebellum

Brain uptake is rapid and probably dependent on blood flow.

Brain uptake depends on blood flow and single pass extraction.

$$K_1 = \text{rate brain entry}$$
$$K_1 = \text{flow} \cdot \text{extraction}$$
$$K_1 = F \cdot E$$

Example

Flow of drug 100 μg per min

Extraction is 2%

$$K_1 = 2 \mu\text{g per min}$$

Single Pass Extraction of [¹¹C]dLop >50%

- 1) Measure K_1 from brain and plasma data of [¹¹C]dLop
- 2) Measure blood flow with [¹⁵O]H₂O
- 3) Calculate Extraction (E)

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

$$K_1 > 0.25 \text{ mL per cm}^3 \text{ per min}$$

$$F = 0.5 \text{ mL per cm}^3 \text{ per min}$$

$$E > 0.5 = 50\%$$

After correction for relative blood flow, [^{11}C]dLop uptake is uniform among brain regions

FC=Frontal Cortex, AC=Anterior Cingulate Gyrus, TE=Temporal Cortex , PA=Parietal Cortex,
HP=Hippocampus, OC= Occipital Cortex, PU=Putamen, CE=Cerebellum

Conclusion

After P-gp blockade, single pass uptake of [¹¹C]dLop into brain is high and, therefore, shows dependence on blood flow

Implies function of P-gp at baseline is rapid and has high capacity

[¹¹C]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?

PET

FUSED

MRI

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

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

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

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

3 most common:

- ABCB1 (P-gp)**
- ABCC1**
- ABCG2**

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

Major problem with previous substrate radiotracers of P-gp: lack of selectivity

Radiotracer	ABCB1 (P-gp)	ABCC1	ABCG2
[99mTc]Sestamibi	Yes	Yes	No
[11C]Verapamil	Yes	Yes	No
[11C]dLop	Yes	?	?

Cultured cell lines from human tumors

**Each ABC transporter is exclusively expressed
by one resistant cell line**

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

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

1. [¹¹C]dLop is retained (trapped) in human brain

2. [¹¹C]dLop is not displaceable in monkey brain

DCPQ 16 mg/kg, Naloxone 5 mg/kg (30 min after injection)

Structure of dLop: weak base

Hypothesis: lysosomal trapping

Competition with other weak bases

**Lysotracker Red: fluorescent weak base
that accumulates in lysosomes**

Displacement of LysoTracker Red
by other weak bases

Accumulation of [³H]dLop blocked by weak bases

Tariquidar has two effects on cell uptake of [³H]dLop
1) Parental cells: inhibit uptake by displacing from vesicles

Tariquidar has two effects on cell uptake of [³H]dLop

- 1) Parental cells: inhibit uptake by displacing from vesicles
- 2) ABCB1 resistant cells: increase uptake by blocking P-gp

Conclusions

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

2) dLop (a weak base) is trapped within acidic lysosomes displaced by other weak bases – and some P-gp inhibitors are weak bases

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

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 [¹¹C]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