Positron Emission Tomographic (PET) Imaging of Efflux Transporters

Robert B. Innis, MD, PhD
Molecular Imaging Branch
NIMH

Outline of Talk

PET: high sensitivity and specificity

Many PET ligands already exist to measure density of transporters – e.g., dopamine transporter in Parkinson disease

P-gp: efflux transporter "protects" organs like brain and testis from some toxins and drugs

[^{11}C]loperamide: avid P-gp substrate but has radiometabolite; measures function

[^{11}C]desmethyl-loperamide (dLop): metabolite is better than parent

After P-gp blockade, [^{11}C]dLop has high brain uptake that is dependent on flow

[^{11}C]dLop in humans: no brain uptake at baseline and slightly increased by P-blockade

Imaging of neuroreceptors by PET

Flow Chart showing isotope production via cyclotron, radio chemistry on precursor to generate ^{11}C-ligand, and positron camera to image ligand distribution in brain

Positron Emission Tomography

Simon R. Cherry, PhD.
Center for Molecular and Genomic Imaging
University of California – Davis

emgi
UC Davis

NIH Rodent PET Camera
^{18}F bone uptake rat

PET image of rat skeleton

Developed By: Mike Green & Jurgen Seidel

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

Radioligand = Drug + Radioactivity

Drug administered at tracer doses

No pharm effects
Labels <1% receptors
Labeled subset reflects entire population

Radioligand disposed like all drugs

Metabolism & distribution

Radiation exposure

Dopamine Transporter: Located on DA Terminals Removes DA from Synapse

Graphic illustration of a dopaminergic neuronal synapse.

SPECT Imaging of Dopamine Transporter in Caudate and Putamen of Human Brain

SPECT Imaging compared to MRI imaging of human brain.

^{123}I-β-CIT Dopamine Transporter SPECT: Decreased in Parkinson's Disease

Comparison between images of a healthy brain and a brain with Parkinson Stage 1

Serial Dopamine Transporter Imaging in a Parkinsons Patient

Comparison of images at baseline with 22 months, 34 months and 46 months

Institute for Neurodegenerative Disorders

PET Imaging to Monitor Embryonic Stem Cell Treatment of "Parkinson Disease" in Rats

Comparison of PET images and uptake in normal rats and rats with unilateral brain lesion, outcome of embryonic stem cells transplant and overlapping PET & MRI

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

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

MRI compared with Wild Type and KO mice.

Graph showing Conc Activity (%SUV) over time (min)

Injection of [11C]Loperamide in P-gp Knockout and Wild Type Mice

Chart comparing concentration (%SUV) with % Brain Activity of radiochemical species

Five P-gp KO and five WT mice were killed 30 min after injection of [^{11}C]loperamide

PROBLEM of [^{11}C]Loperamide Radiometabolite (desmethyl) enters brain

Chemical structure of [^{11}C] Loperamide

Solution: Remove the nonradioactive methyl group

[^{11}C]Desmethyl-loperamide: Better radioligand?
Demethylation product does not enter brain

Chemical Structure

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

Comparison of MRI and PET images in WT, and P-gp KO mice

Graph showing Conc Activity (%SUV) over time (min)

Injection of [11C]*N*-desmethyl-Loperamide in P-gp Knockout and Wild Type Mice

Chart

Three P-gp KO and three WT mice were killed 30 min after i.v. injection of [11C]dLop

DCPQ or Tariquidar Increases Brain Uptake of Radioactivity in Monkey Given [^{11}C]Loperamide

Graph showing radioactivity concentration (%SUV) over time after injection (min)

[^{11}C]dLop in Monkey Brain

Images of baseline (of brain and pituitary) compared to P-gp blocked

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

Graph of radioactivity concentration (%SUV) over time after injection (min) in the following:

Frontal cortex
Anteior cingulated
Temporal cortex
Parietal cortex
Hippocampus
Occipital cortex
Putamen
Cerebellum

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

Is P-gp function uniformly distributed in brain?

Graph showing concentration (%SUV) over time for FC, AC, TE, PA, HP, OC, PU, and CE

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.

Graph comparing baseline with DCPQ

Concentration (%SUV) over time (min)

P-glycoprotein removes lipophilic substrates directly from the plasma membrane

Graphic illustration

Brain uptake depends on blood flow and single pass extraction.

Illustration of blood flow (K1 and k2) into brain

K1 = rate brain entry
K1 = flow · extraction
K1 = F · E

Example
Flow of drug 100 µg per min
Extraction is 2%
K1 = 2 µg per min

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

Measure K1 from brain and plasma data of [11C]dLop

Measure blood flow with [15O]H2O

Calculate Extraction (E)

E = K_1 over F

K_1 > 0.25 mL per cm3 per min
F = 0.5 mL per cm3 per min

E > 0.5 = 50%

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

Graphs comparing concentration (%-SUV) with no flow correction with flow correction over time for FC, AC, TE, PA, HP, OC, PU and CE

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

Conclusions

[^{11}C]dLop: avoids metabolite problem of [^{11}C]loperamide

After P-gp blockade, single pass uptake of [^{11}C]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

Whole body PET images at 3, 20, and 100 minutes after dosing.

P-gp Transport in Human Body

Graphic illustration of P-gp in brain, intestine, liver, lung, kidney, MDR tumor, testis, and placenta.

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

Whole body PET image in human subject.

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

Comparison of PET, Fused PET/MRI, and MRI.

Graph showing Conc radioactivity (%SUV) over time after injection (min) (comparing whole brain with whole brain – vascular corrected)

Graph showing plasma composition (%) over time after injection (min) comparing parent and radiometabolites)

What is it?

PET, fused PET/MRI and MRI images of human brain

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

PET image of same in human brain

DCPQ or Tariquidar Increases Brain Uptake of Radioactivity in Monkey Given [^{11}C]Loperamide

Graph showing radioactivity concentration (%SUV) over time after injection (min).

Renal Cell Carcinoma:
Tariquidar increases uptake of 99mTc-Sestamibi
in metastasis of thigh

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

Future Directions

BRAIN: Potential dysfunction of P-gp at blood-brain barrier: Alzheimer's disease, Parkinson's disease, epilepsy

ONCOLOGY: P-gp function in tumor cells transplanted into mice

Develop radiolabeled inhibitor to measure density, rather than function, of P-gp

Outline of Talk

PET: high sensitivity and specificity

Many PET ligands already exist to measure density of transporters – e.g., dopamine transporter in Parkinson disease

P-gp: efflux transporter "protects" organs like brain and testis from some toxins and drugs

[^{11}C]loperamide: avid P-gp substrate but has radiometabolite; measures function

[^{11}C]desmethyl-loperamide (dLop): metabolite is better than parent

After P-gp blockade, [^{11}C]dLop has high brain uptake that is dependent on flow

[^{11}C]dLop in humans: no brain uptake at baseline and slightly increased by P-blockade

ACKNOWLEDGMENTS

P-gp Efflux Transporter:
Sami Zoghbi, PhD
Jeih-San Liow, PhD
Nick Seneca, PhD

OVERALL:
Director PET Radiochemistry: Victor Pike
Radiochemist: Neva Lazarova
Metabolism: Sami Zoghbi
Rodent Imaging & Image Analysis: Jeih-San Liow
Monkey Imaging: Robert Gladding
Human Imaging: Ferraris Araneta, NP; Chuck Kreisl, MD
Chemistry: Cheryl Morse, Jinsoo Hong, and Kelly Sprague

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.