Positron Emission Tomography: Tool to Study Pharmacokinetics and to Facilitate Drug Development

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National Institute Mental Health

Outline of Talk
1. PET has high sensitivity and specificity
2. PET used in therapeutic drug development
3. Pharmacokinetic modeling of plasma concentration and tissue uptake can measure receptor density
4. Study drug distribution: block distribution to periphery and increase distribution to brain
5. Study drug metabolism: inhibit defluorination

Imaging Receptors with PET
**PET vs. MRI**

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

**Radioligand = Drug + Radioactivity**

1. **Drug administered at tracer doses**
   a) No pharm effects
   b) Labels <1% receptors
   c) Labeled subset reflects entire population
2. **Radioligand disposed like all drugs**
   a) Metabolism & distribution
3. **Radiation exposure**
NIH Rodent PET Camera
\(^{18}\text{F} \) bone uptake rat

Developed By: Mike Green & Jurgen Seidel

PET: Tool in Therapeutic Drug Development

- Determine dose and dosing interval
- Identify homogeneous group
- Biomarker for drug efficacy
- Monitor gene or stem cell therapy

Lazabemide blocks \(^{13}\text{C}\)deprenyl binding to monoamine-oxidase-B (MAO-B)

Selegilene is more potent and longer acting than lazabemide
PET: Tool in Therapeutic Drug Development

• Determine dose and dosing interval
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• Biomarker for drug efficacy
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Dopamine Transporter: Located on DA Terminals
Removes DA from Synapse

SPECT Imaging of Dopamine Transporter in Caudate and Putamen of Human Brain
**PET: Tool in Therapeutic Drug Development**

- Determine dose and dosing interval
- Identify homogeneous group
- Biomarker for drug efficacy
- Monitor gene or stem cell therapy

**Serial Dopamine Transporter Imaging in a Parkinson Patient**
PET Imaging of Amyloid: Biomarker for Alzheimer’s Disease

- PET: Tool in Therapeutic Drug Development
  - Determine dose and dosing interval
  - Identify homogeneous group
  - Biomarker for drug efficacy
  - Monitor gene or stem cell therapy

Gene Therapy Using Viral Vectors

Viral vectors deliver gene that synthesizes dopamine (DA)
Infuse virus into striatum (target cells)

Target cells express the DA gene
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Brain Uptake of $[^{18}F]$Fluoxetine: Measures Density of Serotonin Transporters & Affinity of Fluoxetine

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<th>Patient</th>
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<tr>
<td>AUC=32</td>
<td>AUC=16</td>
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Brain Drug

| Time |

Inject Activity

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<td>20 mCi</td>
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<td>20 mCi</td>
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<tr>
<td>Weight</td>
<td>50 kg</td>
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Liver disease | Yes | No |

Liver disease is associated with reduced Fluoxetine uptake.
Binding Potential (BP): Receptor Density * Affinity

BP equals uptake in brain relative to how much drug is delivered via arterial plasma.

\[
BP = \frac{\text{Area Brain Curve}}{\text{Area Plasma Curve}}
\]

\[
BP = \frac{16}{2} = 8
\]

**Binding Potential: Independent of Injected Dose**

Double Plasma Input => Double Brain Response

*If ligand does not saturate receptors - i.e., if tracer doses used

\[
\text{BP 1st Time} = \frac{16}{2} = 8
\]

\[
\text{BP 2nd Time} = \frac{32}{4} = 8
\]

BP can be calculated from the Area Under Curve (math integral) as well as rate constants (math differential).

From curves of plasma and brain radioactivity over time, estimate rate constants of entry and removal to/from tissue.

\[
BP = \frac{K_1}{k_2}
\]
Tissue uptake is proportional to density of receptors and the affinity of the drug

\[
BP = \frac{B_{\text{max}}}{K_D} = \frac{B_{\text{max}}}{K_D} \times \frac{1}{K_D} = B_{\text{max}} \times \text{affinity}
\]

- \(B_{\text{max}}\) = receptor density
- \(K_D\) = dissociation binding constant
- \(\frac{1}{K_D}\) = binding affinity drug

SUMMARY PET KINETICS

- Organ uptake is proportional to receptor density and affinity of drug
- Binding Potential (BP) = density \(\times\) affinity
- "Drug Exposure" to tissue is AUC of: plasma concentration vs. time
- "Response" (uptake) of tissue is AUC of: tissue concentration vs. time

\[
BP = \frac{\text{Response}}{\text{Exposure}} = \frac{\text{AUC}_{\text{tissue}}}{\text{AUC}_{\text{plasma}}}
\]

- BP also equals ratio of rate constants of entry and removal to/from tissue

\[
BP = \frac{K_1}{k_2}
\]

Major Point of PET Pharmacokinetics (in words)

- Plasma pharmacokinetics provides a limited view of what’s happening to drug in plasma.
- PET provides a limited view of what’s happening to drug in tissue.
- Concurrent measurement of drug in plasma and of drug in tissue allows quantitation of the target of drug action – i.e., receptor.
**Outline of Talk**

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**Translocator Protein (18 kDa)**
a.k.a. “peripheral benzodiazepine receptor”

1. Mitochondrial protein highly expressed in macrophages and activated microglia
2. Exists in periphery and brain
3. Multiple potential functions: steroid synthesis, nucleotide transport
4. Distinct from typical benzodiazepine GABA$_A$ receptor in brain
5. Marker for cellular inflammation

**Receptor Blockade [¹¹C]PBR28 in Monkey Brain:** more radioligand in plasma and brain

![Graph showing baseline and receptor blocked conditions]
Receptor blockade displaces from lung & kidney. Drives more to brain but doesn’t bind there.

Incidental Stroke

Original MRI (T1)  
Repeat MRI 8 weeks after PET

PET 6 weeks after MRI

Repeat MRI (FLAIR, edema)

TSPO identifies epileptogenic focus in 15 of 16 patients.

Hirvonen et al., JNM, 2012
Human with low uptake is similar to monkey with receptor blockade

A) regular healthy subject
B) odd healthy subject
C) normal monkey
D) pre-blocked monkey

No Binding to $^{11}$C]PBR28 in Brain and Periphery

Normal Binding

No Binding (~10% subjects)

TSPO rs6971 polymorphism causes differential affinity for PBR28

- Ala to Thr substitution
- Allelic frequency ~ 30%.
  - Prevalence of homozygotes ~ 9%
- Codominant expression
  - HAB - high affinity binding
  - LAB - low affinity binding
  - MAB - reduced binding (mixed affinity states)

Owen, JCBFM 2012
**Brain Uptake of $[^{18}\text{F}]$Fluoxetine:**

Measures Density of Serotonin Transporters

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<td>100 kg</td>
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<td>Liver disease</td>
<td>Yes</td>
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**Binding Potential (BP): Receptor Density $\times$ Affinity**

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<th>Plasma Drug</th>
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<tbody>
<tr>
<td>Time</td>
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</tr>
<tr>
<td>AUC=16</td>
<td>AUC=2</td>
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BP = $\frac{\text{Area Brain Curve}}{\text{Area Plasma Curve}}$

BP = $\frac{16}{2} = 8$

**Experimental Design: Effect of TSPO genotype on PBR28 binding**

- PET study
  - 27 healthy volunteers
  - In vitro binding: Leukocyte displacement assay
  - In vivo binding: $[^{11}\text{C}]$PBR28 PET imaging

- Post-mortem study
  - 47 healthy controls, 45 schizophrenia patients
  - Specific $[^{3}\text{H}]$PBR28 binding in prefrontal cortex
  - Comparison with and without genotype correction

Kreisl, JCBFM 2013
**PET Study: Both TSPO genotype and leukocyte binding assay determine affinity status**

100% agreement between binding assay results and genotype

One-site fit = HAB

Two-site fit = MAB

Kreisl, JCBFM 2013
Owen, JCBFM 2012

**PET Study: [\(^{11}\text{C}\)]PBR28 binding is 1.4-fold higher in high affinity binders than mixed affinity binders**

Mean HAB = 4.5 mL • cm\(^{-3}\)
Mean MAB = 3.2 mL • cm\(^{-3}\)

Expect less than 2-fold difference because [\(^{11}\text{C}\)]PBR28 uptake represents specific and nonspecific binding

**PET Study: Greater brain uptake in HH subjects with similar plasma concentration as HL subjects**
Post-mortem study: High and mixed affinity binders also seen in schizophrenia patients

Kreisl, JCBFM, 2013

Post-mortem study: Correcting for TSPO genotype increases ability to detect difference in schizophrenia and controls

Without genotype as covariate $p = 0.085$

With genotype as covariate $p = 0.011$

Summary

- PBR28 PET study:
  - Leukocyte binding assay predicts TSPO genotype
  - TSPO genotype influences $[^{11}C]PBR28$ total binding

- PBR28 Post-mortem study:
  - TSPO genotype influences specific binding
  - Genotype correction increases ability to measure difference in schizophrenia and controls

- Correcting for TSPO genotype expected to improve clinical use of $[^{11}C]PBR28$
**TSPO imaging in Alzheimer’s disease:**
William Kreisl, MD *(Brain, 2013)*

Neuroinflammation a proposed contributor to Alzheimer’s disease pathology

- Unclear if early or late phenomenon

<table>
<thead>
<tr>
<th></th>
<th>AD</th>
<th>MCI</th>
<th>Healthy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (n)</td>
<td>19</td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td>Age</td>
<td>63 ± 9</td>
<td>73 ± 10</td>
<td>63 ± 6</td>
</tr>
<tr>
<td>MMSE</td>
<td>20 ± 4</td>
<td>28 ± 2</td>
<td>30 ± 0.4</td>
</tr>
<tr>
<td>Amyloid</td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
</tr>
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</table>

Results: $[^{11}C]PBR28$ uptake increased in Alzheimer’s disease but not mild cognitive impairment

$[^{11}C]PBR28$ uptake increased in Alzheimer’s disease in target but not background regions
[¹¹C]PBR28 binding greater in Alzheimer’s in target regions after correcting for TSPO genotype

- Inf parietal cortex:
  - AD: $p = 0.001$
  - MCI: $p = 1.000$
  - HC: $p = 0.009$

- Cerebellum:
  - AD: $p = 0.204$
  - MCI: $p = 0.004$
  - HC: $p = 1.000$

[¹¹C]PBR28 binding greater in target regions after correcting for TSPO genotype.

- Inf parietal cortex
- Cerebellum

[¹¹C]PBR28 binding correlates with clinical severity across Alzheimer’s disease spectrum

- $r = 0.590$
- $p = 0.001$


Early onset AD patients have greater [¹¹C]PBR28 binding

- EOAD: $p = 0.009$
- LOAD:

Early onset AD patients have greater [¹¹C]PBR28 binding.
Conclusions from Alzheimer’s disease study

• Neuroinflammation occurs after conversion of MCI to AD and worsens with disease progression

• Neuroinflammation greater in early onset patients
  – May explain precipitous course in early onset patients

• $[^{11}\text{C}]$PBR28 has promise for longitudinal study of AD
  – Mark conversion from MCI to AD
  – Assess response to treatments

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$[^{18}\text{F}]$FCWAY: Defluorination
Bone uptake: human skull at 2 h
**[¹⁸F]FCWAY: Defluorination**

¹⁸F-fluoride ion accumulates in bone

![Chemical Structure](image)

**Miconazole Inhibits Defluorination & Bone Uptake**

![Images of [¹⁸F]Fluoride and [¹⁸F]FCWAY: Miconazole with baseline and Miconazole doses (15 mg/kg, 30 mg/kg, 60 mg/kg)]

**Disulfiram: Decreases Skull Activity & Increases Brain Uptake**

![Images of Baseline and Disulfiram uptake](image)

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

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Self-Assessment Quiz:
True or False?

• Imaging with positron emission tomography (PET) involves the injection of a radioactively labeled drug that emits a particle called a positron.
• PET shows the location of radioactivity in a cross section (or tomograph) of the body.
• PET can be used to quantify the density of specific proteins in the body.
• Compartmental modeling of PET data typically uses measurements over time of 1) PET images of the target tissue and 2) concentrations of unchanged parent radioligand in plasma.

FDA Critical Path Initiative

• Approvals for new drugs declining
• R&D funding by industry and NIH is increasing
• Problem: tools are inadequate for efficient evaluation of new drugs in the “critical path” of development
• Still using old tools like liver enzymes and hematocrit to evaluate safety and efficacy
• Need new Product Development Toolkit
“There is currently an urgent need for additional public-private collaborative work on applying technologies such as … new imaging technologies.

Opportunity: Imaging technologies, such as molecular imaging tools in neuropsychiatric diseases or as measures of drug absorption and distribution, may provide powerful insights into the distribution, binding, and other biological effects of pharmaceuticals.”
Quantification of receptor density

**Distribution volume**

Uptake in brain relative to how much drug is delivered via arterial plasma

\[
V_T = \frac{\text{Area Brain Curve}}{\text{Area Plasma Curve}}
\]

\[
V_T = \frac{16}{2} = 8
\]

- **Brain Drug**
- **Plasma Drug**

Time after injection

---

Quantification of receptor density

**Equilibrium method**

Distribution volume

Concentration ratio of tissue to plasma under equilibrium

- **Bolus injection**
- **Equilibrium method**

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<th>Time after injection</th>
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<td>Plasma Drug</td>
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Advantages of **equilibrium method**

- Determine VT directly from concentration ratio of tissue to plasma under equilibrium
- Less invasive
- Rapid equilibrium can be achieved with bolus and constant infusion
Rapid equilibrium with bolus plus constant infusion

Radioactivity became stable in plasma and brain with bolus plus constant infusion