Translational Molecular Imaging: Infection/Inflammation

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Translational Molecular Imaging

• Translational MI: bringing experimental therapies/diagnostics to the clinic, after extensive testing in animal models.
  – Assess safety, lack of side-effects in humans
  – Test for clinical utility/ usefulness in clinical trials
  – Evaluate for advantage(s) over existing techniques/agents
Preclinical research → Translational research → Clinical application

- Unacceptable side effects and/or immunological response
- No benefit over current methods
- Complex labeling
- High radiation
- Prohibitive cost
Translational MI: Infection/Inflammation

• “Ideal” infection/inflammation imaging agent:
  – Accumulation/retention at site of infection
  – Low background uptake: clearance from blood pool and major organs mainly bowels
  – Early rather than delayed imaging protocol
  – Low radiation dose, easy labeling, low cost
  – No dependence on host leukocytes

  – Ability to differentiate infection from inflammation
Molecular targets in Infection/inflammation

- Inflammation +/- infection:
  - Vasodilation/Increased blood flow
  - Increased vascular permeability
  - Migration of leukocytes to inflammatory focus (chemotaxis)
  - *Presence of micro-organisms (infection)
Histamine
serotonin
Complement system
Activation
Activated Kinin system
Activation
Chemokines/cytokines
AA metabolites
Histamine
serotonin
Complement system
Activation
Kinin system
Activation
Increased blood supply
and vascular permeability
Release of mediators
Histamine
serotonin
Complement system
Activation
Kinin system
Activation
Chemokines
cytokines
Histamine
serotonin
AA metabolites
Inflammation
WBC margination, rolling, adhesion, emigration
Increased blood supply and vascular permeability
Release of mediators

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Histamine
serotonin
Complement system
Activation
Kinin system
Activation
Chemokines
cytokines

Histamine
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AA metabolites

WBC margination, rolling,
adhesion, emigration
Increased blood supply
and vascular permeability
Release of mediators

Inflammation

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Molecular imaging targets in inflammation/infection

- Increased blood supply
  - vascular permeability
    - $^{67}$Ga-citrate,
    - Human polyclonal immunoglobulins
    - Liposomes
  - Endothelial cell activation
    - F(ab’)$_2$-anti-E-selectin
- Increased metabolism
  - $^{18}$F-FDG
- Presence of micro-organism
  - Labeled antibiotics (Infecton)
  - Antimicrobial peptides
- Infiltrating leukocytes
  - $^{99m}$Tc-Anti-SSEA-1 (leuTech)
  - $^{99m}$Tc-anti-NCA-90 Fab’ (LeukoScan)
  - Labeled IL-8, LTB4-antagonists
Increased permeability

- Agents leaking through endothelium:
  - Non specific e.g. $^{67}$Ga-citrate

$^{99m}$Tc-PEG-Liposomes in patients with Crohn’s disease

Unacceptable side effects: shortness of breath, increased BP, erythema of face and upper extremities, tachycardia

[Brouwers et al., J Drug Target, 2000]
Leukocyte targeting

- Labeled leukocyte scan: gold standard
- Problem: *In vitro* labeling
- Solution: *In vivo* labeling
  - Antigen binding:
    - NCA-90 surface antigen
    - CD15 antigens
  - Receptor binding:
    - Cytokines: IL-8 targets CXCR-1 and CXCR-2
Leukocyte targeting: NCA-90 binding

$^{99m}$Tc-monoclonal Fab’ fragments (sulesomab) LeukoScan®

Soft tissue infections: pneumonia, infected skin ulcer

[Quigley et al., Med Princ Pract, 2008]
Leukocyte targeting: NCA-90 binding

$^{99m}$Tc-monoclonal Fab’ fragments (sulesomab) LeukoScan®

[modified from Iyengar et al., Nuc Med Commun. 2005]
Leukocyte targeting: CD-15 binding

$^{99m}$Tc-labeled antistage specific embryonic antigen-1 (antiSSEA-1) NeutroSpec®

Approved by FDA in July 2004
Withdrawal from market December 2005

Leukocyte targeting: Cytokine Receptors

- $^{99m}$Tc-IL-8 in 20 patients:
  - 10 true positives
  - 2 false negative
  - 8 true negatives

- No appreciable side effects

Larger scale studies needed

Liver Abscess

[Bleeker-Rovers et al., JNM, 2007]
Leukocyte targeting

• Leukocyte imaging limitations:
  – Non specific for infection
  – Decreased usefulness in immunosuppressed patients
Microorganism targeting

- Labeled Antibiotics
- Labeled antimicrobial peptides
- Enzymatic substrates of bacterial enzymes
Labeled antibiotics

• $^{99m}$Tc-ciprofloxacin (Infecton®)
  – Excreted by kidneys
  – Low liver metabolism \(\rightarrow\) low bowel activity
  – Known mechanism of action of the antibiotic
  – No bone marrow uptake \(\rightarrow\) osteomyelitis imaging

**Assumption:** when labeled with $^{99m}$Tc, it keeps most of the pharmacodynamic and pharmacokinetic properties of the unlabeled antibiotic \(\rightarrow\) binds living bacteria
99mTc-Ciprofloxacin (Infecton)

Infected left hip prosthesis

[Larrika et al., Nucl Med Commun, 2002]
Labeled antibiotics

• 879 pts scanned with Infecton®:
  – sensitivity 85.4%
  – specificity 81.7%

• Contradictory results from different groups:
  “99mTc-Ciprofloxacin cannot differentiate infection from inflammation”
$^{99m}$Tc-ciprofloxacin scintigraphy in prosthetic knees.

Chemical structure unknown: could Technetium be changing the binding capacity of the drug?
Labeled antibiotics

- $^{18}$F-Ciprofloxacin was synthesized and tested

**Conclusion:** “…non-specific binding rather than specific binding to bacterial type II topoisomerase enzymes is the predominant mechanism of bacterial retention of the radiotracer. … the kinetics of $^{18}$F ciprofloxacin in infected tissue are governed by increased blood flow and vascular permeability rather than by a binding process.”

Molecular imaging targets in inflammation/infection

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  - Human immunoglobulins
  - Liposomes

- Endothelial cell activation
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- Presence of micro-organism
  - Labeled antibiotics (Infecton)
  - Antimicrobial peptides
  - Enzyme substrates

- Increased metabolism
  - $^{18}$F-FDG

- Infiltrating leukocytes:
  - $^{99m}$Tc-Anti-SSEA-1 (leuTech)
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  - (LeukoScan)
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Antimicrobial peptides

- Natural protective role → cationic particles that interact with bacterial cell wall components such as phospholipids.

- One derivative of Ubiquicidin, UBI 29-41, has been applied in humans with promising results.
Radiolabeled FIAU

- FIAU substrate of the viral thymidine kinase (HSV-1\(tk\)), used extensively in reporter gene imaging

- Incidentally discovered to act as a substrate for the bacterial TK
Bacteriolytic therapy of tumors

Anaerobes home to the hypoxic core and proliferate: *bacteriolytic* therapy

(Courtesy of Chetan Bettegowda and Martin Pomper)
Bacteriolytic therapy of tumors

If bacteria can be killed by FIAU → they can be imaged by radiolabeled-FIAU.

(Courtesy of Chetan Bettegowda and Martin Pomper)
Mechanism of FIAU Uptake

To test whether thymidine kinase mediates sensitivity to FIAU, TK null *E. coli* were generated.

[Bettegowda C et al., PNAS, 2005]

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Radiolabeled FIAU

• Bacterial Thymidine Kinase shares enough similarity to HSV-1\textit{tk} to allow imaging using radiolabeled FIAU

• Multiple bacteria evaluated: all susceptible to FIAU
Human Translation

- Translation relatively quick:
  - FIAU structure well defined
  - Tracer dose of FIAU (300,000 x less than the no effect-dose) are used → no risk of liver failure
  - Previous human use of the radiolabeled nucleoside
(a) septic arthritis (right knee),
(b) septic arthritis (right knee),
(c) osteomyelitis (left distal tibia),
(d) cellulitis (left lower extremity),
(e) necrotizing septic arthritis (left knee).

124I-FIAU-PET/CT

[Diaz LA et al., PLoS ONE, 2007]
Translational MI: Infection/Inflammation

- All of these examples mostly apply to extracranial organ systems
- In the CNS, infection and inflammation imaging is different

Blood-Brain-Barrier
Blood-Brain-Barrier

Astrocytic endfeet

Pericytes

Basement membrane

Tightly apposed endothelial cells - no gaps

Tight junctions

BRAIN

CAPILLARY

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Neuroinflammation

- Neuroinflammation: common denominator in multiple CNS diseases
  - Neurodegenerative: AD, parkinson, huntington’s, ALS, CJD…
  - White matter demyelination/ multiple sclerosis
  - AIDS dementia
  - Herpes encephalitis
Inactive microglia

Activated microglia

CNS injury

neurotoxins

Nitric oxide
Eicosanoids
Free radicals
Chemokines: MIP-1α, MCP-1
Cytokines: IL-1, IL-6, TNFα

Cellular neuronal damage
Apoptosis

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Activated microglia

CNS injury
Activation

Mitochondria

PBR/TSP

↑ number of PBR/TSP
↑ number of mitochondria

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Peripheral Benzodiazepine Receptor/Translocator protein

TSPO ligand

$^{11}$C-PK11195
$^{11}$C-DAA1106
$^{11}$C-DPA-713
$^{11}$C-PBR28
$^{18}$F-FEPPA

Outer Mitochondrial membrane

Voltage dependent anion channel

Inner Mitochondrial membrane

Translocator protein

Adenine nucleotide transporter

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Neuroinflammation in Alzheimer’s Disease
$[^{11}C](R)$-PK11195

[Cagnin et al., Lancet, 2001]
Neuroinflammation in AD

\[^{11}C\](R)-PK11195

Okello et al., Neurology, 2009

MCI-I: these normal PK binding and MCI-II has increased

SNM
2009 Annual Meeting
Neuroinflammation in AD

$[^{11}C](R)$-PK11195

[Wiley et al, Arch Neurol, 2009]
Neuroinflammation in HIV-Associated Dementia

$[^{11}\text{C}](R)$-PK11195

[Slides are not to be reproduced without permission of author.]

[Hammoud et al., J Neurovirol, 2005]
Neuroinflammation in Multiple sclerosis

\[^{11}\text{C}]\text{(R)-PK11195}\]

[Debruyn et al., Eur J Neurol. 2003]
Future prospects

- Need agents that work in immunosuppressed as well as immuno-competent patients
- Different agents for different clinical situations
- $^{99m}$Tc issues: need alternative radionuclides while keeping radiation exposure low
- MR translational molecular imaging applications