Peptides, Nanoparticles and other New Cardiac Radiopharmaceuticals

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Background

- Cardiovascular Disease remains the leading cause of death in the Western world. Atherosclerosis is the usual cause of heart attacks, strokes and peripheral vascular disease.
- With coronary artery disease, plaque grows within the walls of the coronary arteries until the blood flow to the heart is limited - causing ischemia.
- Atherosclerosis is a chronic inflammatory process elicited by hyperlipidemia.
- Myocardial infarction & sudden cardiac death can result from rupture of vulnerable atherosclerotic plaques.
- Myocardial perfusion imaging can determine reversible ischemia and characterize infarcted tissue.
Challenges in Clinical Diagnosis

- Need for early diagnosis is critical since atherosclerosis is a silent disease that progresses slowly
- PET imaging could characterize atherosclerotic plaques with high sensitivity allowing risk stratification
- Co-registration with CT or MR can combine sensitivity with increased resolution
- Currently the most widely studied PET probe for detection of plaque-based inflammation is $^{18}$F-FDG, taken up by active macrophages
- FDG is a nonspecific imaging probe for inflammation and also accumulates in calcified structures
Imaging PET Isotopes
Cyclotron and Generator Produced

\[ ^{64}\text{Ni}(p,n)^{64}\text{Cu} \quad t_{1/2} = 12.7 \text{ hours} \]

\[ ^{18}\text{O}(p,n)^{18}\text{F} \quad t_{1/2} = 109.7 \text{ min.} \]

\[ ^{68}\text{Ge}/^{68}\text{Ga Generator} \quad t_{1/2} = 68 \text{ min} \]
Areas of Focus

• Atherosclerotic plaque formation and detection
  – Naturetic peptides and receptors
  – Chemokine receptors as markers of inflammatory cell influx
    • Atheroviral macrophage inflammatory Protein-II (vMIP-II) based imaging probe
    • D-Alpha1-peptide T-amide (DAPTA) peptide based tracers for detection of CCR5 receptor expressed on plaque

• Myocardial perfusion imaging (MPI)
  – For determination of reversible ischemia, infarcted tissue
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Natriuretic Peptides and Receptors: New Target for Atherosclerosis Imaging

- Natriuretic peptides (NP) ANP, BNP – involved in homeostasis and natriuresis
- BNP increases in heart failure
- In 1990, CNP became the third member of the natriuretic peptide family to be discovered (Sudoh T, et al. *Biochem Biophys Res Commun* 1990)
- CNP found in high concentrations in atherosclerotic lesions and has anti-migratory effects on vascular smooth muscle cells (VSMCs)


C-ANF$_{4-18}$: a Selective Fragment for the NPR-C

- The full length C-type natriuretic peptide is vasoactive, however, a fragment of CNP, C-ANF (C-type Atrial Natriuretic Factor) is not.
- NPR-C (receptor) is upregulated in atherosclerotic plaques
- Investigate potential of C-ANF to image plaque-like lesions
- Used a rabbit model-mimics human atherosclerosis
- Conjugated the C-ANF to DOTA chelate, labeled with $^{64}$Cu

$^{64}$Cu-DOTA-C-ANF$_{4-18}$:

$^{64}$Cu-DOTA-Arg-Ser-Ser-c[Cys-Phe-Gly-Gly-Arg-Ile-Asp-Arg-Ile-Gly-Ala-Cys]-NH$_2$
64Cu-DOTA-C-ANF Conjugated Nanoprobe Targeting Angiogenesis

- Demonstrated ability to image upregulation NPR-C expressed on atherosclerotic plaques using 64Cu-DOTA-C-ANF
- Next develop CANF-integrated nanoprobe to prove the presence of NPR-C during angiogenesis
- Using mouse model of hind limb ischemia (HLI) induced angiogenesis
Methods: Synthesis of CANF-Comb nanoparticles

- **Nanoparticle** CANF-Comb
  - **Size**: 22 nm
  - **Zeta potential**: -1.1±2 mV
  - **Octanol/water coefficient**: -1.3
  - **CANF amount**: 35/particle
  - **MW/PDI**: 210 kDa/1.2
  - **No. of DOTA**: 70
  - **$^{64}$Cu labeling specific activity**: 20 mCi/nmol

Hawker C, Pressly E, UCSB
$^{64}$Cu-radiotracers (20 µCi) were injected IV into Hind Limb Ischemia (HLI) mouse model, expressing NPR-C in injured limb. Imaged 7 days after surgery, shows markedly higher SUV ratio for $^{64}$Cu-CANF-Comb in comparison to $^{64}$Cu-CANF alone. Dynamic scan 0-60 min.

Accelerated Atherosclerosis Model with Wire Injury

• Induced in ApoE\(^{-/-}\) mice, 6 week old
• Fed Western diet (42% fat) 2 weeks
• Isolated the femoral artery branch, small incision made in vessel
• Guide wire advanced 2 cm in the descending aorta
• Wire left in place 2 min to induce endothelial damage
• Left femoral artery, with incision & without guide wire injury, served as sham site

Control Mice:
• Normal Wild Type (WT) C57BL/6 mice
• Fed normal chow
• Underwent same wire-injury
$^{64}\text{Cu-CANF-Comb}$ targeting NPRC receptor in Apo E$^{-/-}$ atherosclerosis model

All mice: 24 h post injection
Apo E$^{-/-}$ mice: 20 weeks old on Western diet
RT-PCR: NPRC Expression in Aortas

Relative Expression of NPR-C in ApoE-/ mice on high cholesterol diet vs. C57 mice on normal diet

Validation/Optimization Studies in Multiple Pre-Clinical Models

Cell Studies

- No uptake in NPR-A and NPR-B expressing cells

Saturation Binding Assay
- \( K_d = 0.95 \pm 0.20 \) nM
- \( B_{max} = 2236 \pm 199 \) fmol/mg
- Receptors/cell: \( 5.4 \times 10^6 \)

Athero/Vascular Injury: Rabbits

Gene Expression

Liu et al., JNM 2010;51:85

HLI-Angiogenesis: Mouse

BioD

Liu et al., JNM 2011;52:1966
$^{64}$Cu-CANF-comb Targeting NPRC vs. $^{18}$F-FDG PET In Rabbit Model

* n=10  
** n=3

p < 0.0001  
p < 0.0001  
p < 0.001  
p < 0.001
PET/MR for Carotid Imaging: Protocol optimization using $^{18}$F-FDG

- $N = 18$
- $^{18}$F-FDG 444-703 MBq
- PET: 15 min list mode acquisition;
- PET reconstruction: 3D OSEM
- MR: T1, PD, T2, 3D T2 SPACE;
  2-point Dixon for AC
- MR resolution: $0.25 \times 0.25 \times 0.8$ mm; dedicated carotid coil
- PET resolution (HD): 4 mm

Lau JM, Laforest R, Woodard PK et al. RSNA, 2014
Human PET-MRI w/ $^{64}\text{Cu-25\% CANF-Comb}$

4.0 mCi injection, imaged at 24 hours, 30 minute acquisition

- PET: 30 min list mode acquisition;
- PET reconstruction: 3D OSEM
- MR: T1, PD, T2, 3D T2 SPACE; 2-point Dixon for AC
- MR resolution: 0.25 x 0.25 x 0.8mm; dedicated carotid coil
- PET resolution (HD): 4 mm

Diseased right common carotid artery
Non-diseased left common carotid
cervical spine
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Targeting Chemokine Receptors in Atherosclerosis

Chemokines:

- Signaling proteins secreted by cells
- Can induce directed chemotaxis in responsive cells
- Family of small, soluble chemotactic cytokines expressed by a host of different cells including endothelial cells (EC) and smooth muscle cells (SMC)
- Divided into 4 groups based on spacing of 1st two cysteine residues: CX; CC; CXC; CX₃C;
- Thought to contribute to inflammation by cell recruitment (in a number of disease states)

Chemokine receptors:

- 7 transmembrane structure that couples to G-protein for signal transduction
- Usually bind more than one chemokine
- Expressed on EC, SMC, and circulating leukocytes
Viral Macrophage Inflammatory Protein-II (vMIP-II) based imaging probe

Viral Macrophage Inflammatory Protein-II (vMIP-II)

- v-MIP-II is a 71 amino acid Kaposi sarcoma-associated herpes virus protein that is a molecular mimic of CCL2 which recruits cells to site of inflammation.
- The virus uses vMIP-II to enter inflammatory cells and block host immune responses
- It binds and antagonizes wide range of chemokine receptors present on inflammatory cells important in the development of atherosclerosis: CCR1, CCR2, CCR3, CCR4, CCR5, CCR8, CXCR3, CXCR4, XCR1, and CX3CR1
- Indicates potential to determine atherosclerosis progression

vMIP-II based imaging probes

- $^{64}$Cu-DOTA-vMIP-II
- $^{64}$Cu-DOTA-vMIP-II-Comb

ApoE$^{-/-}$ mouse spontaneous atherosclerosis model

- High cholesterol diet starting from 6 weeks
- Longitudinal study up to 37 weeks
- Age-matched wild type mouse as control
**64Cu-DOTA-vMIP-II preparation**

- **vMIP-II** (15 nmol) + DOTA-NHS (180 nmol) → PBS, pH 7.5, Overnight, 4°C → DOTA-vMIP-II → 64Cu-DOTA-vMIP-II

1. 64CuCl2, 0.1M NH4OAc, pH 5.5, 37°C, 1 hr
2. EDTA, 10 min
3. SEC purification (Zeba)

**64Cu radiolabeling specific activity:** 200μCi/μg

- **Biodistribution:** 5μCi of 64CuDOTA-vMIP-2 (n=4)
- ApoE−/− mice model, 4 weeks post wire injury
- 1 h post injection

Accelerated murine atherosclerosis model

$^{64}$Cu-DOTA-\(\text{vMIP-II}\) PET/CT

ApoE\(^{-/-}\) mice induced atherosclerosis with wire injury

$^{64}\text{Cu-vMIP-II-Comb}$

**Preparation and Biodistribution**

$^{64}$Cu-vMIP-II-Comb imaging atherosclerosis in a mouse ApoE$^{-/-}$ model

Macrophage staining (F4/80, brown) demonstrated increased plaque size and macrophage positive area along the progression of plaque.

Reverse transcription polymerase chain reaction (RT-PCR) analysis demonstrated increased expression of 8 chemokine receptors at RNA level, consistent with the PET data.

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Chemokine receptor CCR5

- Chemokine CCL5 (RANTES), upregulated in human atherosclerotic lesions, mainly modulates the inflammatory process through CCR1,3,5.

- CCR5 plays a central role in promoting late stage plaque. It is identified in advanced plaque and closely associated with plaque stability.

- CCR5 antagonist treatment in ApoE^{-/-} mice showed decreased plaque size and attenuated monocyte/macrophage infiltration.

$^{64}$Cu-DOTA-DAPTA PET/CT in vascular injury induced atherosclerosis model.

64Cu-DOTA-DAPTA-Comb Synthesis and Biodistribution

$^{64}$Cu-DOTA-DAPTA-Comb PET/CT in vascular injury induced atherosclerosis model

$^{64}$Cu-DOTA-DAPTA-Comb PET/CT in ApoE$^{-/-}$ + Wire Injury model, demonstrated specific detection of CCR5 receptor.

The upregulation of chemokine receptors in mouse ApoE\(^{-/-}\) wire injury atherosclerosis model has been demonstrated via immunohistochemistry (IHC) and reverse transcription polymerase chain reaction (RT-PCR), especially at late stage of disease.

Consistent with RT-PCR data, IC staining showed increased macrophage burden in plaque with disease progression.

The vMIP-II based peptide and nanoparticle radiotracers showed specific detection of a group of chemokine receptors in ApoE\(^{-/-}\) mice, showing their potential to determine macrophage burden and plaque progression.

The DAPTA peptide based PET tracers showed specific detection of CCR5 receptor expressed on plaque. RT-PCR data showed consistent pattern to PET results in the longitudinal study, indicating the potential of \(^{64}\text{Cu-DO}-\text{DAPTA}\) and \(^{64}\text{Cu-DO}-\text{DAPTA-Comb}\) to determine atherosclerosis activity.

**Summary**

- The upregulation of chemokine receptors in mouse ApoE\(^{-/-}\) wire injury atherosclerosis model has been demonstrated via immunohistochemistry (IHC) and reverse transcription polymerase chain reaction (RT-PCR), especially at late stage of disease.
- Consistent with RT-PCR data, IC staining showed increased macrophage burden in plaque with disease progression.
- The vMIP-II based peptide and nanoparticle radiotracers showed specific detection of a group of chemokine receptors in ApoE\(^{-/-}\) mice, showing their potential to determine macrophage burden and plaque progression.
- The DAPTA peptide based PET tracers showed specific detection of CCR5 receptor expressed on plaque. RT-PCR data showed consistent pattern to PET results in the longitudinal study, indicating the potential of \(^{64}\text{Cu-DO}-\text{DAPTA}\) and \(^{64}\text{Cu-DO}-\text{DAPTA-Comb}\) to determine atherosclerosis activity.
Chemokine CCR2 Antagonism in Atherosclerosis

Millennium Announces MLN1202 Fully Met Primary Endpoint in Phase II Clinical Study of Patients at High Risk for Atherosclerosis

Novel CCR2 antagonist shows statistically significant and sustained clinical activity

CAMBRIDGE, Mass., May 31 /PRNewswire-FirstCall/ -- Millennium Pharmaceuticals, Inc. (Nasdaq: MLNM) today announced positive top-line results from the Company's randomized, double-blinded, placebo-controlled Phase II clinical trial of MLN1202, a novel humanized monoclonal antibody that specifically targets the CCR2 chemokine receptor in patients at high risk for atherosclerotic cardiovascular disease. Preliminary analysis showed that MLN1202 was well tolerated and fully met its primary endpoint of a significant reduction in C-Reactive Protein (CRP) levels, an inflammatory biomarker associated with atherosclerosis, for months after a single dose of MLN1202. These results were statistically and clinically significant relative to the placebo control arm (p = 0.0275). No serious adverse events were observed in patients exposed to MLN1202. Trial results are expected to be submitted for presentation at a medical meeting.
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ABC transporters are transmembrane proteins that utilize the energy of adenosine triphosphate (ATP) hydrolysis to carry out certain biological processes.

Galmydar is a membrane potential probe, following penetration, it localizes within mitochondria of myocardium cells.

Chemical Structure

http://sharmalab.wustl.edu/
PET Myocardial Perfusion Imaging

$^{68}$Ga-[3-isopropoxy-ENDBMPI] ($^{68}$Galmydar)

- Reacted with Ga-68 chloride in 0.01% acetylacetone in ethanol
- Heated at 100°C for 10 min, HPLC
- Uptake in myocytes
- Rapid clearance from the liver
- Excretion from kidneys

Vijay Sharma, Sivapacklam J, et al:
[www.plosone.org](http://www.plosone.org); Oct 2014: Vol 0 Issue 10
$^{68}$Galmydar: PET Imaging of Normal Myocardium in Rat and Rabbit Models

RAT Myocardium

[Image of Rat Myocardium with MicroPET/CT; 60 min Post Injection]

Rabbit Myocardium

[Image of Rabbit Myocardium with Axial, Coronal, and Sagittal views at 30 min, 60 min, and 120 min post injection]

http://sharmalab.wustl.edu/
Conclusion

New radiolabeled cardiac PET tracers including peptides, nanoparticles and small molecules, may increase ability to detect and evaluate atherosclerotic lesions as well as provide a Ga-68 perfusion imaging agent.
Thank-you to the Washington University Investigators who provided slides for the presentation

- Pamela Woodard, MD
- Yongjian Liu, PhD
- Hannah Luehman, MS
- Vijay Sharma, PhD
Thank you!
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