Copper 64-Cu Nanoparticles in Multimodal Imaging of Carotid Artery Disease

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ABSTRACT

Nanoparticle based hybrid imaging by MRI, PET-CT and Fluorescent reflectance techniques are emerging in imaging carotid artery disease. 64Cu-TNP is detectable by MRI and PET-CT. A trimodality reporter 64Cu-TNP is a derivatization product made of: 1. the chelator DTPA attached with radiotracer 64Cu and iron oxide core in center provides contrast in MRI imaging(T2, T2*, or steady-state free-precession sequences); 2. fluorochrome attached with 64Cu-TNP is used for fluorescence imaging, including fluorescence microscopy, flow cytometry, and fluorescence-mediated tomography; 3. crosslinked aminated polysaccharide coating provides the biocompatibility and determines the blood half-life and provides linker for attachment of tracers and potentially affinity ligands. The novelty of 64-Cu nanoparticle is that it provides structural, molecular and physiological information same time in carotid artery disease. Other possible techniques based 64-Cu nanoparticles are possible multiple contrast magnetic resonance imaging, susceptibility weighted imaging, positron emission tomography, nanoparticle based imaging, computer tomography, fluorescent based imaging, and fluorescent microscopy but not confirmed yet.
Key words: Cu-64 nanoparticle, atherosclerosis, bioimaging, multimodal imaging

1 INTRODUCTION

Major applications may be possible as in vivo imaging molecular readouts by optical, MRI, SPECT modalities; in vivo imaging targeted diagnostics/ therapeutics of carotid artery atherosclerosis [1]. For vascular imaging applications, we observed potentials of liposome vesicles (50-70 nm) in US and MRI, perfluorocarbon core emulsions (200-300 nm) for MRI, US, fluorescence, nuclear, CTI, HDL, LDL micelles for MRI.

2 MOLECULAR IMAGING OF CAROTID ARTERY PLAQUES: HOW IT WORKS?

Molecular imaging of atherosclerosis offers in vivo biology insight as well as new clinically translatable strategy to identify and classify high risk carotid artery plaques. Rapid growth of optical, near-infrared fluorescence, molecular imaging are making possible to get chemical and molecular events in plaque inflammation and angiogenesis with possibility of clinical intervention such as intravascular catheters, noninvasive tomography as achievable dream of single platform multimodal imaging. The development of multimodal, multifunctional nanoparticles to image carotid artery is growing science in understanding and treating vascular disease.

How nanoparticles generate contrast?

Suppose two tissues have same T1 and T2 relaxation constants. After nanoparticle injection, only tissue A expresses the molecular epitope that binds the paramagnetic particles. The paramagnetic particles affect the relaxation constants as following:

$$1/T_{1A} = 1/T_{1B} + r_1[NP] \quad (2)$$

$$1/T_{2A}=1/T_{2B}+r_2\,[NP] \quad (3)$$

,where T_{1B} and T_{2A} are unchanged relxation constants while $r_1= 1/T_{1A}$ and $r_2 = 1/T_{2A}$ are observed relaxation constants after nanoparticle binding. The r_1 and r_2 are relativities of nanoparticles calculated by slope from plot r_2 vs concentration C* of nanoparticle.

$r_1 = y = A*[1-\exp^{(-r1*TR)}]$; and

$r_2 = A+C*[\exp^{(-r2*TE)}]$

For SE pulse sequence, MR signal intensities of each tissue will be:

$$S_A=k(1-2e^{-(TR-TE/2)/T1A} + e^{-TR/T1A})e^{-TE/T2A} \quad (4)$$

$$S_B=k(1-2e^{-TR-TE/2)/T1B} + e^{-TR/T1B})e^{-TE/T2B} \quad (5)$$

where k1, and k2 are scan intrinsic properties such as flip angle, coil sensitivity, proton density etc.

$$CNR = |(S_A-S_B)/N| \quad (6)$$

Optimizing TR is suitable technique to generate highest CNR of two tissues at different T1 relaxation constants.

$$TR_{opt}=T_{1A}T_{1B}/(T_{1B}-T_{1A})\ \ln(k_AT_{1B}/k_BT_{1A} \quad (7)$$

Over several years, our lab has got a library of nanoparticles with bioimaging and therapeutic application potentials. For molecular imaging, our strategy was make 10-50 nm sized nanoparticles for vascular endothelium tissue targeting and functionalization (binding with drug, antibodies, peptides, polysaccharides, avidin-biotin cross-linked with polymers) suited for in vivo targeting.

3 TRIMODAL DMOLECULAR IMAGING: COPPER-IRON NANOPARTICLES

Molecular probes offer the potential to characterize biochemical features by targeting of biochemical epitopes such as perfluorocarbon nanoparticles are echogenic for US, usable for MRI, CTI, SPECT imaging of thrombosis and angiogenesis. Author proposes a new multi-labeling technique for multimodal imaging such as iron-Cu, iron-saccharaide, iron-gold,iron-Lanthanum or gadolinium. Copper has caught attention bound with dextran coating around the nanoparticle

crosslinked with epichlorin hydrin aminated and labeled with near-infrared fluorochrome Vivotag-680 first reported with following possibilities and implications of multimodal molecular imaging [3].

- Derivatization with the chelator DTPA to attach with radiotracer ^{64}Cu.
- Iron oxide core provided contrast in MRI (T2, T2*, or steady-state free-precession sequences).
- Fluorochrome for fluorescence imaging, fluorescence microscopy, flow cytometry, and fluorescence-mediated tomography.
- Crosslinked aminated polysaccharide coating for biocompatibility, determined blood half-life, and provided linker for attachment of tracers and potentially affinity ligands.
- ^{18}FDG PET for hybrid PET-CT Imaging on X-PET PET-CT system (Mercury Computer Systems, Carlsbad, Calif).
- MRI Microimaging Studies on 7-T horizontal-bore scanner (Bruker Pharmascan, Billerica, Mass).
- In Vivo Fluorescence Reflectance Imaging, Fluorescence Microscopy, Phosphorimaging. Autoradiography, Flow Cytometry using triple fluorescent labeled imaged with an upright epifluorescence microscope (Eclipse 80i, Nikon, Melville, NY)
- Histopathology to compare the detectable regions and morphology.

4 SYNTHESIS OF ^{64}CU-TNP

Nanoparticle MION with dextran coating was crosslinked with epichlorin hydrin, aminated and labeled with near infrared fluorochrome Vivotag-680 (VT680, VisEn Medical, Woburn, MA) in ratio of VT680 per nanoparticle (5 dye moieties/NP).

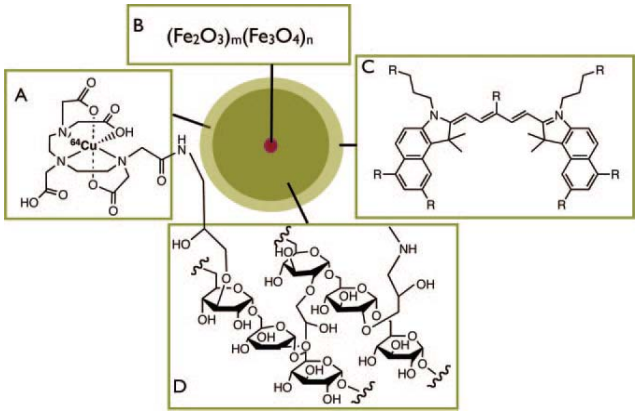

Figure 1. Cu-64 TNP. Schematic view of the trimodality reporter Cu-64 TNP. A, Derivatization with the chelator DTPA allows attachment of radiotracer 64Cu. B, Iron oxide core provides contrast in MRI (T2, T2*, or steady-state free-precession) sequences). C, Fluorochrome for fluorescence imaging, including fluorescence microscopy, flow cytometry, and fluorescence-mediated tomography. D, Crosslinked aminated polysaccharide coating provides biocompatibility, determines blood half-life, and provides linker for attachment of tracers and potentially affinity ligands.Reproduced with permission reference [2].

In second step, nanocomplex gets mixed with excess dianhydride DTPA (Sigma, St. Louis, MO) for 2 hours in 0.1M borate buffer, pH 9.3 at room temperature in phosphate buffer (pH 7.4) for the preparation of ^{64}Cu-DTPA-NP or ^{64}Cu-TNP). Non-radioactive copper salts.

Using this strategy, Nerendorff et al. modified nanoparticles further as following:

1.One hundred µg DTPA-NP labeling with $^{64}CuCl_2$ (IsoTrace, Toronto, Canada) or 185 MBq ^{64}Cu, in ammonium acetate buffer (180 µL, 0.5 M, pH 5.5);

2.After 25 min of incubation at 95°C, content centrifugation and washing three times to get pure ^{64}Cu-TNP to re-dissolve in 400 µL PBS;

3.Routine analysis of aliquots by HPLC (eluents: A= 0.1% TFA in water and B= acetonitrile; gradient: 0-20 min, 95%-40% A; 20-24 min, 95% B; 24-28 min, 95% B; 30 min, 95% A), using a Varian 210 HPLC (Salt Lake City, UT) with a C_{18} column, multi-wavelength detector and a flow-through gamma-detector. The specific activity of ^{64}Cu-TNP was 1 mCi per 0.1 mg Fe of NP (corresponding to approximately 300 µCi/mouse or 1.5 mg Fe/kg bodyweight);

4.The average diameter measurement of the NP was 20 nm by laser light scattering; 5.The measurement of R1 and R2 values were approximately 29 and 60 mMsec-1 (0.47T, 39°C).

5 ^{64}Cu-TNP IS DETECTABLE BY MRI AND PET-CT

Nirendorff et al. [2] detected threshold of the nanoparticle in the imaging phantom as sufficient 5 µg Fe/mL using T2 weighted MRI, and 0.1 µg Fe/mL for PET-CT imaging as shown in Figure 2. The dose requirement of nanoparticles is within safe limits. Nanoparticles are quite sensitive to PET.

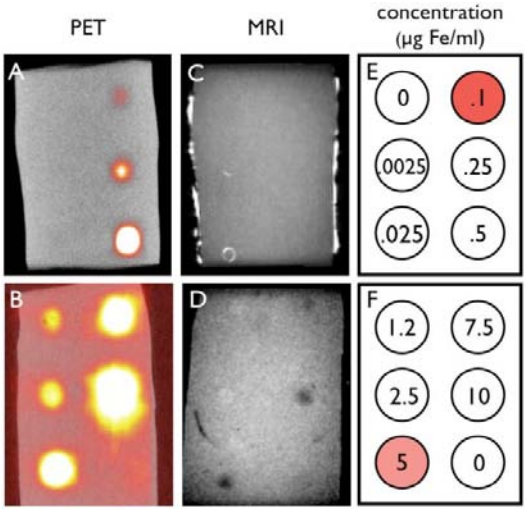

Figure 2. Detection threshold for 64Cu-TNP by MRI and PET. Two agar phantoms with increasing concentration of 64Cu-TNP were first imaged by PET-CT (A and B), followed by T2*weighted gradient-echo MRI at 7 T (echo time, 140 ms; C and D). E and F, The concentrations of nanoparticles in wells. In the current experimental setup, the lowest concentration detected by MRI was 5 _g Fe/mL agar and by PET was 0.1 _g Fe/mL, 50 times lower than MRI. Reproduced with permission from Reference [2].

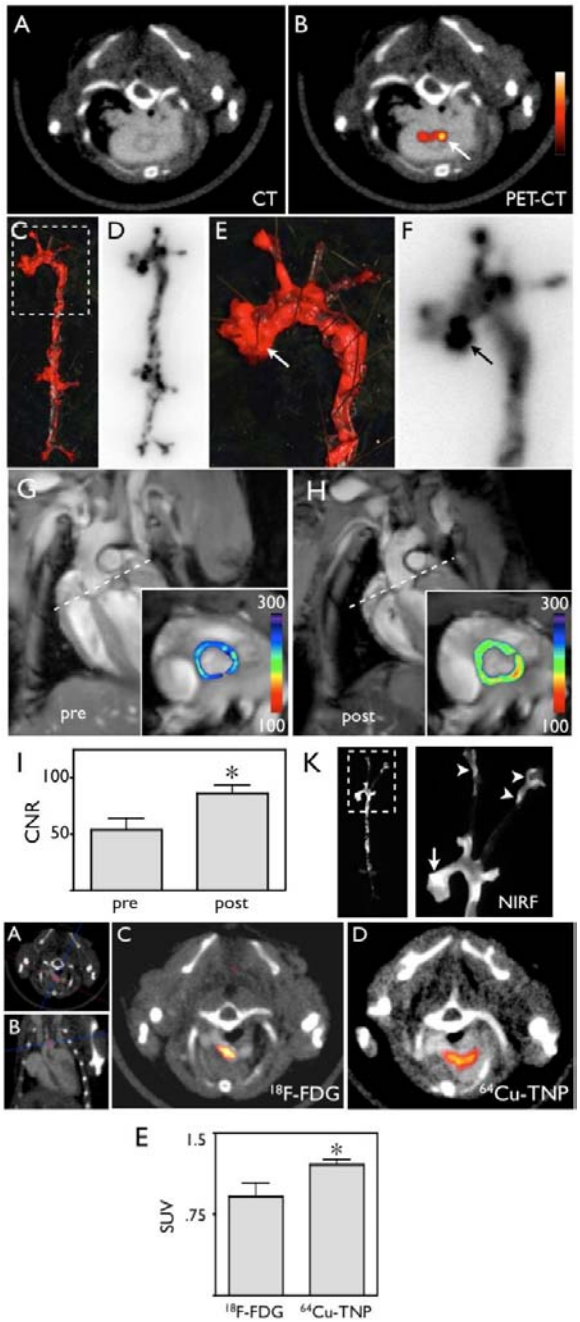

Figure 5. 64Cu-TNP distributes to atherosclerotic lesions. A and B, PET-CT shows enhancement of the posterior aortic root (arrow). C through F, Enface Oil Red O staining of the excised aorta depicts plaque-loaded vessel segments, which colocalize with areas of high 64Cu-TNP uptake on autoradiography. E and F, Zoomed image of the root and arch. Arrows depict a plaque-laden segment of the root with high activity, which corresponds to the in vivo signal seen in B. G through I, Preinjection and postinjection MRIs of the aortic root (inset). The dotted line in the long-axis views demonstrates slice orientation for short-axis root imaging. I, Signal intensity (pseudocolored with identical scaling for preinjection and postinjection image) decreased significantly after injection of 64Cu-TNP, which was quantified by calculation of the contrast-to-noise ratio (CNR). K, Near-infrared fluorescence reflectance imaging (NIRF) of excised aortas shows accumulation of the probe in plaques residing in the root (arrow), thoracic aorta, and carotid bifurcation (arrowheads), further corroborating the PET signal observed in these vascular territories. *P_0.01.Reproduced with permission from reference [2].

6 NEW LESSONS OF 64CU NANOPARTICLES

Phagocytic activity and inflammatory activity in atherosclerosis plaque can be detectable and measurable by trimodal hydrid imaging. 64Cu-polymer encaged iron oxide nanoparticles. Newly introduced hybrid synthesis seems future of nanoelement applications such as copper, gold, silver, iron, cadmium, manganese, zinc, iodine, fluorine, phosphorus etc with paramagnetic rare earth elements such as lanthanum, gadolinium has potential in making nanocomposites encaged with polymer coats and further can be tagged with specific antibodies. Such hybrid approach can give opportunity to visualize the odd numbered nuclei(MRI), radiotags(PET, SPECT), photons(CT), acoustic waves(US, PTI), bioluminescence or photophosphoresence (FTI, OCT, NIRF) as part of nanoparticles for imaging purpose. The sensitivity, diagnostic accuracy, absolute concentration measurement, specificity of molecular label to the tissue disease entity will remain a open issue to more investigations and explorations. Safety of using nanoparticles, optimal dose administration within safe limits with risk free imaging applications is still an open controversy. It will require extensive research to make these nanoparticles useful as imaging modality.

7 CONCLUSION

Copper element can be used in imaging. Both non-radioactive and radioactive copper forms can be used in imaging. Cardiovascular imaging is emerging a clinical modality more aggressively by use of nanoparticles synthesized using iron oxide linked with copper-64-DTPA encaged in polymers. Multimodal imaging by nanoparticles is a new art to generate simultaneously different molecules of cardiovascular tissue by MRI,PET,CT,SPECT,US or molecular imaging by NIRF. Still the use of these new nanoelement based techniques are in infancy and research is needed for their successful use in clinical imaging.

8 REFERENCES

1. Sharma R. et al. http://www.scribd.com/doc/24988334/Evaluation-of-Carotid-Atherosclerosis-Plaques
2. Nahrendorf M et al. Circulation. 2008 January 22; 117(3): 379–387.