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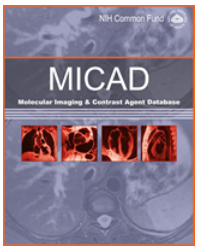

^{64}Cu-DTPA-CLIO-VT680

^{64}Cu-TNP

Huiming Zhang, PhD[1]

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Chemical name:	^{64}Cu-DTPA-CLIO-VT680	
Abbreviated name:	^{64}Cu-TNP	
Synonym:	^{64}Cu trireporter nanoparticle	
Agent Category:		
Target:	Macrophages	
Target Category:	Phagocytosis	
Method of detection:	Magnetic resonance imaging (MRI), optical, positron emission tomography (PET)	
Source of signal/contrast:	Iron oxides, VT680, ^{64}Cu	
Activation:	No	
Studies:	• *In vitro* • Rodents	No structure is available in PubChem.

Background

[PubMed]

Macrophages are key cellular mediators of inflammation in atheroma and participate in all phases of atherogenesis (1, 2). The atheroma lesion is initialized by recruiting monocytes in inflamed intima. Monocytes mature into macrophages under the *in situ* stimulation of overexpressed macrophage colony-stimulating factor. As cholesteryl esters gradually accumulate in cytoplasm, macrophages are converted into foam cells at the early stage of atheroma. The accumulation of foam cells leads to the formation of fatty streaks and the deposition of fibrous tissues, indicating the progression of atheroma into an intermediate stage. Fibrous caps are formed on the surface of a lipid-rich core and result in vulnerable plaque as the smooth muscle cells synthesize bulk extracellular matrix. The rupture of plaque and the calcification of vessel walls progressively occlude the lumen. All of these developmental stages are produced in apolipoprotein E–deficient (apoE$^{-/-}$) mice (3), a transgenic animal model with targeted deletion of the apoE gene. As a ligand, apoE binds to the receptors that are responsible for clearing chylomicrons and very low density lipoprotein remnants (4). A deficiency in apoE reduces diet cholesterol absorption and leads to a substantial increase in plasma cholesterol levels. ApoE$^{-/-}$ mice

Author Affiliation: 1 National Center for Biotechnology Information, NLM, NIH, Bethesda, MD; Email: micad@ncbi.nlm.nih.gov.

are used in the study of spontaneous hypercholesterolemia and the subsequent development of atherosclerotic lesions (4, 5). The histopathological progression found in apoE$^{-/-}$ mice is very similar to that found in humans (3). Because the arch shape and the proximity to three major arteries (the right and left common carotid arteries and the left subclavian arteries), atherosclerosis primarily occurs at the aortic arch in apoE$^{-/-}$ mice (3).

Dextran-coated paramagnetic nanoparticles, such as ultra-small superparamagnetic iron oxide particles (USPIO), are known to accumulate in the macrophages located in inflamed lesions and carotid artery plaques after intravenous administration (6). The active internalization mechanism may be associated with dextran receptor–mediated endocytosis (1). Intracellular dextranase cleaves the dextran coating and leaves the iron oxide to be solubilized into iron ions followed by progressive incorporation into the hemoglobin pool (6). Because of long circulation times, excellent biocompatibility, and high relaxivity, USPIO are widely used to enhance magnetic resonance imaging (MRI) contrast for imaging lesional macrophages in stroke, multiple sclerosis, atherosclerotic diseases, spinal cord injury, and brain tumors (6). Monocrystalline iron oxide nanoparticles (MION) are a special type of USPIO that contain an icosahedral core of superparamagnetic crystalline Fe_3O_4 (magnetite) with a diameter of ~5 nm (7). Using epichlorohydrin to cross-link the dextran coating and amine groups to functionalize the surface, MION can be converted into an aminated platform (cross-linked iron oxide (CLIO)-NH_2) that allows attachment of various imaging probes (8). CLIO-NH_2 carrying fluorescent probes has demonstrated preferential uptake by lesional macrophages, concomitant with a much lower uptake by endothelial cells and smooth muscle cells (1).

^{64}Cu-Labeled diethylenetriamine pentaacetic acid (DTPA)-CLIO-Vivotag 680 (VT680) is a ^{64}Cu-labeled triple reporter nanoparticle (^{64}Cu-TNP) used in MRI, positron-emission tomography (PET), and optical fluorescence imaging (9). ^{64}Cu-TNP consists of three probe types. A CLIO-NH_2 forms the core of the nanoparticle, generates a T_2-shortening effect in MRI, and is recognizable by macrophages. Numerous complexes of ^{64}Cu-DTPA are attached to the surface of CLIO-NH_2 for PET detection. ^{64}Cu is a positron-emitting radionuclide with an intermediate half-life (12.7 h) that decays by positron (β^+) with a branching factor of 17.4% and a maximum β^+ energy of 0.653 MeV (10). ^{64}Cu has been used as a radiotracer in PET imaging and a radiotherapy agent in cancer treatment. Five molecules of VT680, an amine-reactive N-hydroxysuccinimide (NHS) ester of a (benzyl)-indolium–derived far-red fluorescent probe that remains internalized in cells for days without interfering with cell functions, are attached to CLIO-NH_2 for fluorescence imaging (11). Its excitation/emission peak is located at 670 ± 5 nm/688 ± 5 nm. The internalization mechanism includes diffusion into cells within minutes and covalent binding to cellular components (11). This macrophage-targeted PET/MRI/optical agent allows complementary information to be obtained by multimodal imaging (9). The PET tracer is detected at 10^{-6}–10^{-8} M (10), providing a sensitivity to detection that is at least one order of magnitude higher than the 10^{-5} M in MRI (9). The spatial resolution of MRI permits mapping of the atherosclerotic vascular territories. The cell-specified optical probes explore the fate of the imaging probe at cellular levels (9, 11).

Synthesis

[PubMed]

Nahrendorf et al. described a detailed synthesis of ^{64}Cu-TNP (9). As the core of the triple reporter agent, CLIO-NH_2 was synthesized in several steps (12). The starting material MION was synthesized by neutralization of ferrous salts, ferric salts, and dextran with ammonium hydroxide, followed by ultra-filtration. The obtained MION was cross-linked in a strong base with epichlorohydrin and then reacted with ammonia to produce CLIO-NH_2. CLIO-NH_2 then was reacted with the NHS ester of VT680 (commercially available) followed by incubation with excess dianhydride DTPA. Finally, the purified product was reacted with 185 MBq (0.5 mCi) $^{64}CuCl_2$. For MRI experiments, stable nonradioactive copper salts ($CuCl_2$) were used to produce Cu-TNP. ^{64}Cu-TNP had a hydrodynamic diameter of ~20 nm and was attached with five VT680 moieties per

nanoparticle. The specific activity of ^{64}Cu-TNP was determined to be 1 mCi per 0.1 mg Fe of the nanoparticles, corresponding to ~300 μCi per mouse at a dose of 1.5 mg Fe/kg.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The relaxation efficacy of Cu-TNP was measured at 0.47 T and 39°C (9). The T_1 and T_2 relaxivity values were found to be 29 and 60 $mM^{-1}s^{-1}$, respectively. The detectable threshold of Cu-TNP in MRI or PET was examined in an agar phantom (9) in which ^{64}Cu-TNP was diluted in 2% agarose to concentrations ranging from 0.0025–10 mg Fe/ml. The phantom was imaged with a combined PET/computed tomography (CT) scan followed by MRI at 7 T. The detection threshold of ^{64}Cu-TNP was found to be 0.1 μg Fe/ml in PET and 5 μg Fe/ml in MRI.

The efficiency of fluorescent labeling was examined in excised aortas with *ex vivo* fluorescence reflectance imaging at 700 nm (9). Mice were killed after MRI to obtain aorta tissue samples. With a magnification of ×2, the plaque target/background ratio was found to be 8.0 ± 1.9 in the aortic root, arch, and carotid arteries of ApoE$^{-/-}$ mice. The excised aortas of apoE$^{-/-}$ mice were digested in cell suspensions for flow cytometry study to determine the uptake of ^{64}Cu-TNP at cellular levels (9). Fluorescein isothiocyanate–labeled monoclonal antibodies were used to identify macrophages, neutrophils, smooth muscle cells, and endothelial cells. Macrophage-associated activity was found to contribute 73.9% of the overall activity; other cells such as neutrophils, endothelial cells, lymphocytes, and smooth muscle cells also ingested ^{64}Cu-TNP and collectively contributed the remaining signal activity (~26%). The aortic roots of apoE$^{-/-}$ mice were also stained with hematoxylin and eosin for immunofluorescence imaging of cell nuclei (9). With an upright epifluorescence microscope, fluorescence images were obtained in three spectrally resolved channels: ultraviolet for 4',6-diamidino-2-phenylindole (DAPI) nuclear staining, Texas red for immunofluorescent cell-specific antibodies, and far-red for VT680. The primary accumulation of ^{64}Cu-TNP appeared to be within macrophages, as demonstrated by co-localization with MAC-3 antigen. Some uptake also occurred in lesional smooth muscle cells adjacent to macrophage-rich regions of highly inflamed plaques and in endothelial cells.

Animal Studies

Rodents

[PubMed]

The blood half-life of ^{64}Cu-TNP was measured in wild-type B6 mice (n=5) (9). Repetitive retro-orbital bleeds were collected at 0.5, 1, 2, 6, and 24 h after intravenous injection of 150 μCi ^{64}Cu-TNP. The mean blood half-life of ^{64}Cu-TNP was found to be 259 ± 39 min. The distribution ^{64}Cu-TNP in various organs was examined 24 h after injection; at this time point, 81.3 ± 3.3% of activity (decay-corrected) remained in the animal. The percent injected dose per gram of tissue was 33.6 ± 7.3 in the liver, 15.8 ± 2.8 in the small intestine, 13.8 ± 1.8 in the kidney, 11.0 ± 2.5 in the lung, 9.4 ± 3.6 in the spleen, 6.0 ± 0.9 in the heart, 5.2 ± 0.9 in the aorta, 4.3 ± 1.1 in the lymph nodes, 2.4 ± 1.2 in the thymus, and 1.1 ± 0.3 in fat. In apoE$^{-/-}$ mice, the organ distribution was similar to that found in the wild-type mice, but the percent injected dose was much higher than that found in wild-type mice: 260% in aortas and 392% in carotid arteries. No *in vivo* analysis of free ^{64}Cu was performed.

ApoE$^{-/-}$ mice (45 weeks old) on a 20-week, high-cholesterol diet were imaged with PET/CT(n=9) or MRI (n=5) (9). ApoE$^{-/-}$ mice received intravenous injections of 303 ± 39 μCi ^{64}Cu-TNP at a dose of 1.5 mg Fe/kg and were imaged with PET/CT 24 h later. This dose was approximately one order of magnitude lower than the 30 mg Fe/kg used in MRI. A solution containing 61% Iopamidol (a CT contrast agent) was infused continuously and intravenously at 65 μl/min during the 2-min CT acquisition, which was used to provide appropriate intravascular contrast at a spatial resolution of 72 μm. After completion of CT acquisition, a PET scan was

performed for 30–45 min at a spatial resolution of 2 mm. All imaged apoE$^{-/-}$ mice showed a significant PET signal in the aortic root and arch. The target/background ratio was 5.1 ± 0.9 in the aortic root. ^{18}F-Fluoro-2-deoxy-D-glucose (^{18}F-FDG) PET was used to further confirm the plaques in the aortic root regions. *In vivo* MRI studies were performed at 7 T. ApoE$^{-/-}$ mice were imaged before and 48 h after intravenous injection of 30 mg Fe/kg Cu-TNP, using a gradient-echo fast and low angle shot (FLASH) sequence. A significant contrast enhancement was found in the aortic root 48 h after injection of Cu-TNP. After MRI, *ex vivo* fluorescence reflectance imaging and autoradiography were conducted on excised aortas to confirm the uptake of ^{64}Cu-TNP.

Other Non-Primate Mammals

[PubMed]

No publication is currently available.

Non-Human Primates

[PubMed]

No publication is currently available.

Human Studies

[PubMed]

No publication is currently available.

NIH Support

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