Iron oxide–ferritin nanocages

Fn-Fe nanocages

Kam Leung, PhD[1]

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Chemical name:	Iron oxide–ferritin nanocages	
Abbreviated name:	Fn-Fe nanocages	
Synonym:		
Agent category:	Polypeptide nanoparticle	
Target:	Non-targeted	
Target category:	Non-targeted	
Method of detection:	Magnetic resonance imaging (MRI)	
Source of signal:	Iron oxide	
Activation:	No	
Studies:	• *In vitro* • Rodents	Structure is not available in PubChem.

Background

[PubMed]

Magnetic resonance imaging (MRI) maps information about tissues spatially and functionally. Protons (hydrogen nuclei) are widely used to create images because of their abundance in water molecules, which comprise >80% of most soft tissues. The contrast of proton MRI images depends mainly on the nuclear density (proton spins), the relaxation times of the nuclear magnetization (T1, longitudinal; T2, transverse), the magnetic environment of the tissues, and the blood flow to the tissues. However, insufficient contrast between normal and diseased tissues requires the use of contrast agents. Most contrast agents affect the T1 and T2 relaxation times of the surrounding nuclei, mainly the protons of water. T2* is the spin–spin relaxation time composed of variations from

[1] National Center for Biotechnology Information, NLM, NIH, Bethesda, MD; Email: MICAD@ncbi.nlm.nih.gov.

Corresponding author.

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molecular interactions and intrinsic magnetic heterogeneities of tissues in the magnetic field (1). Cross-linked iron oxide nanoparticles and other iron oxide formulations affect T2 primarily and lead to a decreased signal. On the other hand, paramagnetic T1 agents, such as gadolinium (Gd^{3+}) and manganese (Mn^{2+}), accelerate T1 relaxation and lead to brighter contrast images.

Endothelial cells are important cells in inflammatory responses (2, 3). Bacterial lipopolysaccharides, viruses, inflammation, and tissue injury increase secretion of tumor necrosis factor α (TNFα), interleukin-1 (IL-1), and other cytokines and chemokines. Emigration of leukocytes from blood is dependent on their ability to adhere to endothelial cell surfaces. Inflammatory mediators and cytokines induce chemokine secretion from endothelial cells and other vascular cells and increase their expression of cell-surface adhesion molecules, such as intracellular adhesion molecule-1, vascular cell adhesion molecule-1, integrins, and selectins. Chemokines are chemotactic to inflammatory cells (such as leukocytes and macrophages) attracting them to sites of inflammation and tissue injury. Under atherogenic conditions, deposition of lipids on the endothelial cell surfaces of the aorta and inflammatory cells leads to the development of atherosclerotic plaques (4), which may erode and rupture.

Ferritin (Fn) is composed of 12 or 24 subunits of heavy and/or light chains, which self-assemble to form a cagelike nanoparticle (nanocage) at physiological pH (7.4) with internal and external diameters of 8 nm and 12 nm for the 24 subunit Fn (5-7), respectively. There are four or eight ion channels for directing Fe^{2+} ions to multiple Fe^{2+}/O oxidoreductase ("ferroxidase") sites in the heavy chains for $Fe_2O_3 \bullet H_2O$ deposition in the Fn cavity. The ion channels also control reduction, dissolution, and exit of Fe^{2+} from the mineral with gated pores on the surface of Fn cages. Fe^{2+} ions are required for protein cofactor synthesis and anti-oxidant activity after stress. Most ferritins are intracellular and tissue-specific. For applications, the outer surface of Fn can be chemically or genetically modified with ligands, and the cavity of Fn can capture metal ions with high affinity (8). Uchida et al. (9) loaded iron oxide into the cavities of human heavy chain Fn (Fn-Fe) nanocages to study the *in vitro* uptake of Fn-Fe nanocages by macrophages. Fn-Fe nanocages have been studied for MRI of vascular macrophages in atherosclerotic plaques in mice (10).

Related Resource Links:

- Chapters in MICAD (Ferritin)
- Gene information in NCBI (Ferritin heavy chain, ferritin light chain)
- Articles in Online Mendelian Inheritance in Man (OMIM) (Ferritin heavy chain, ferritin light chain)
- Clinical trials (Ferritin)

Synthesis

[PubMed]

Recombinant human Fn subunits (heavy chains) were purified from transfected *Escherichia coli* cell lysates. For cage mineralization, ammonium iron(II) sulfate hexahydrate (12.5 mM) and hydrogen peroxide (4.17 mM) solutions (7.9 ml each) were added simultaneously to a solution (10 ml) of Fn nanocages (19.8 nmol, pH 8.5) over a period of ~50 min at 65°C (9). After the mineralization procedure, sodium citrate (60 mmol) was added to chelate any remaining free Fe. After dialysis, Fn-Fe nanocages were purified with column chromatography. There were ~3,000-5,000 Fe molecules per nanocage. The diameters of Fn-Fe nanocages were determined with dynamic light scattering and transmission electron microscopy (TEM) to be 13.5 nm and 12 nm, respectively. The average size of iron oxide particles inside the cage was determined with TEM to be 5.9 ± 0.9 nm. R1 and R2 relaxivity values (1.5 T) of Fn-Fe nanocages were 8.4 $mM^{-1}s^{-1}$ and 93 $mM^{-1}s^{-1}$, respectively. These values were similar to those of Ferumoxides and Ferumoxtran-10.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Uchida et al. (9) performed cellular uptake studies of Fn-Fe nanocages (0.165 mg Fe/ml), Ferumoxides (0.165 mg Fe/ml), and Ferumoxtran-10 (0.165 mg Fe/ml) in murine macrophage cells (RAW). Internalization of Fn-Fe nanocages by RAW cells was similar to that of Ferumoxides as measured with Prussian blue staining of Fe after 24 h of incubation, whereas Ferumoxtran-10 showed little staining at the same time point. Quantitative analysis of Fe uptake as measured with inductively coupled plasma mass spectrometry showed that cellular uptake values were 116 pg Fe/cell, 152 pg Fe/cell, and 3 pg Fe/cell at 72 h of incubation for Fn-Fe nanocages, Ferumoxides, and Ferumoxtran-10, respectively. There were slight decreases (~10%) in cell viability of treated RAW cells, but these decreases were not statistically significant ($P > 0.05$).

Animal Studies

Rodents

[PubMed]

Terashima et al. (10) performed MRI (7 T) of macrophages in atherosclerotic carotid arteries in streptozotocin (STZ)-induced diabetic mice. At day 14 after STZ injection, the left common carotid artery (LCA) was ligated in 5 mice and sham-operated in 3 mice. Fn-Fe nanocages (25 mgFe/kg) were injected intravenously. MRI images of both LCA and right common carotid artery (RCA) were obtained at 24 h and 48 h after injection. There was a clear T2* signal loss in the macrophage-rich ligated LCA, but not in the control RCA or in either artery in sham-operated mice. Quantitative analysis showed that the T2*-induced reduction in lumen contrast was significant at both 24 h and 48 h in the ligated LCA compared to contralateral RCA and sham controls ($P < 0.01$). Immunohistochemical analysis showed macrophage infiltration in the ligated LCA but

not in the non-ligated RCA. Fn-Fe nanocages were co-localized with macrophages in the neointima of the ligated LCA but not in the RCA or sham-operated arteries.

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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