Lactoferrin-conjugated superparamagnetic iron oxide nanoparticles

Lf-SPIONs

Liang Shan, PhD[1]

Created: September 25, 2012; Updated: October 24, 2012.

Chemical name:	Lactoferrin-conjugated superparamagnetic iron oxide nanoparticles	
Abbreviated name:	Lf-SPIONs	
Synonym:		
Agent Category:	Nanoparticles	
Target:	Lactoferrin receptors	
Target Category:	Receptors	
Method of detection:	Magnetic resonance imaging (MRI)	
Source of signal / contrast:	Iron oxide	
Activation:	No	
Studies:	• *In vitro* • Rodents	No structure available.

Background

[PubMed]

The lactoferrin (Lf)-conjugated superparamagnetic iron oxide nanoparticles (SPIONs), abbreviated as Lf-SPIONs, was synthesized by Xie et al. for use as a contrast agent for magnetic resonance imaging (MRI) of brain tumors (1).

The blood–brain barrier (BBB) is composed of tight junction-sealed brain capillary endothelial cells (BCECs) and supporting pericytes and astrocytic endfeet (2). Exogenous compounds are prevented by the BBB from reaching the brain by passive transport or

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

Corresponding author.

NLM Citation: Shan L. Lactoferrin-conjugated superparamagnetic iron oxide nanoparticles. 2012 Sep 25 [Updated 2012 Oct 24]. In: Molecular Imaging and Contrast Agent Database (MICAD) [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2004-2013.

through the paracellular route (3). Because many proteins including Lf could effectively cross the BBB through transcytosis, an active transport mechanism of BCECs, this mechanism has been actively used to design brain-targeted delivery systems for targeted imaging and therapy of neurological diseases (4, 5).

Mammalian Lf is a cationic iron-binding glycoprotein (80 kDa), and its receptor expresses on the endothelial cells of the BBB (6). Upon binding with its receptor, Lf could cross the BBB through receptor-mediated transcytosis (5). The transport of Lf across the BBB is unidirectional, from the apical side to the basolateral side, with no apparent intraendothelial degradation (7, 8). Studies with membrane preparations of mouse brain have shown that the Lf receptor in the BCECs has two classes of binding sites: a high-affinity site that has a dissociation constant (K_d) of 10.61 nM and a B_{max} of 410 fmol bound/μg protein; and a low-affinity site that has a K_d of 2,228 nM and a B_{max} of 51,641 fmol bound/μg protein (5). The plasma concentration of endogenous Lf (~5 nM) is lower than the K_d of Lf receptors in the BBB, which avoids the competitive inhibition of endogenous Lf to exogenous Lf-conjugated agents (8). The Lf receptor has also been shown to be overexpressed in various tumors, including brain glioma (1). Because of these features, Lf has been applied as a ligand in designing brain-targeted delivery systems.

Xie et al. developed the contrast agent Lf-SPIONs by conjugating Lf to SPIONs (1), while Qiao et al. developed the agent Fe_3O_4-Lf by conjugating Lf to Fe_3O_4 nanoparticles through polyethylene glycol (8). For the former agent, Lf was designed to target Lf-SPIONs to Lf receptor-expressing tumors. For the latter agent, Lf served to enhance the BBB-penetrating ability of Fe_3O_4-Lf by targeting Lf receptors expressed in the BCECs. Studies with the two agents showed that Lf-SPIONs were able to effectively enhance the glioma contrast, and that Fe_3O_4-Lf could effectively penetrate the BBB in healthy rats because of the conjugation of Lf (1, 8). This chapter summarizes the data obtained by Xie et al. with Lf-SPIONs (1).

Related Resource Links:

Nucleotide and protein of lactoferrin

Protein of lactoferrin receptor

Lactoferrin-related clinical trials in ClinicalTrials.gov

Synthesis

[PubMed]

Xie et al. first modified SPIONs with meso-2,3-dimercaptosuccinic acid and then conjugated to Lf through classic EDC/sulfo-NHS-mediated amidation reaction (1). The final products, Lf-SPIONs, were collected with magnetic separation and dispersed in phosphate-buffered saline. The chemical yield and purity of Fe_3O_4-Lf were not reported.

Lf conjugation was evaluated with polyacrylamide gel electrophoresis and Fourier transform infrared spectroscopy (FTIR). Gel electrophoresis showed the specific band of Lf-SPIONs. FTIR showed the characteristic spectra of Lf-SPIONs, presenting peaks at ~1,668 cm^{-1} for the amide I band and at ~1,562 cm^{-1} for the amide II band. Peaks from bare SPIONs were observed at ~630, 590, and 450 cm^{-1}, typical for Fe–O absorption bands. Lf was estimated to be 0.1 mg/mg SPIONs with the Bradford method. Bare SPIONs and Lf-SPIONs showed similar morphology under transmission electron microscopy (TEM). The size increased from 9.2 ± 0.4 nm for bare SPIONs to 13.6 ± 0.7 nm for Lf-SPIONs. The Lf-SPIONs dispersed in water had a hydrodynamic diameter of 74.8 ± 11.5 nm, larger than the hydrodynamic diameter of the SPIONs (62.0 ± 12.1 nm). The number of Lf per SPION was not reported.

The magnetic properties of SPIONs and Lf-SPIONs at room temperature were measured with vibrating sample magnetometry, exhibiting the saturation magnetization of 51 emu/g Fe for the Lf-SPIONs, lower than 78 emu/g Fe for the bare SPIONs, suggesting that Lf conjugation slightly changed the physical properties of SPIONs. MRI studies on the agent-mixing agarose gel phantoms showed that Lf-SPIONs could reduce the transverse relaxation time T2. With the increase of Lf-SPIONs concentration in the phantom, the signal intensity decreased and the relaxation curves became steeper. The R2 relaxivity coefficient values for Lf-SPIONs and SPIONs were 75.6 $mM^{-1}s^{-1}$ and 131.4 $mM^{-1}s^{-1}$, respectively.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Three cell lines including human embryonic kidney 293 (HEK 293), human normal liver cell line (HL-7702), and human umbilical vein endothelial cell line (ECV 304) were used to test the cell viability after exposure to bare SPIONs or Lf-SPIONs at concentrations of 0, 25, 50, 75, and 100 μg/mL Fe, respectively (1). Both SPIONs and Lf-SPIONs within the concentration range didn't affect the cell viability as demonstrated with MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assays.

Internalization of the particles was measured with fast sequential atomic absorption spectrometry (AAS) and observed under TEM after incubation of C6 glioma cells with SPIONs and Lf-SPIONs at different iron concentrations (5, 10, 15, and 20 μg/mL) at 37°C for 0.5 h. The cellular uptake of Lf-SPIONs presented a dose-dependent, increasing adsorption curve on AAS. Lf-SPIONs accumulated in the cytoplasm of C6 cells on TEM images.

On MRI images (3.0 T) of the cell-embedded agarose phantoms, the signal intensity of C6 glioma cells treated with Lf-SPIONs at 1 μg/mL decreased markedly, whereas only negligible MRI contrast was observed from the control cells and the cells treated with bare SPIONs.

Animal Studies

Rodents

[PubMed]

In vivo MRI studies of Lf-SPIONs were performed with male, brain glioma-bearing Wistar rats ($n = 9$) after injection with SPIONs or Lf-SPIONs (12 mg Fe/kg) (1). High contrast of the brain glioma was observed with Lf-SPIONs between 2 h and 48 h after injection, but MRI with SPIONs showed no visible change over the same period. The decrease of MRI signal intensity in tumors was 36%–74% with Lf-SPIONs, as compared with the pre-contrasted tumors. Histology and Prussian staining of the tumor samples confirmed the presence of significant amount of Lf-SPIONs around vascular region of the tumor tissue at 48 h after injection. At high magnification (500×), Lf-SPIONs were observed to be within the cells. Poor in vivo uptake of SPIONs by the glioma cells was also verified with the same methods.

Other Non-Primate Mammals

[PubMed]

No references are currently available.

Non-Human Primates

[PubMed]

No references are currently available.

Human Studies

[PubMed]

No references are currently available.

References

1. Xie H., Zhu Y., Jiang W., Zhou Q., Yang H., Gu N., Zhang Y., Xu H., Yang X. *Lactoferrin-conjugated superparamagnetic iron oxide nanoparticles as a specific MRI contrast agent for detection of brain glioma in vivo.* Biomaterials. 2011;32(2):495–502. PubMed PMID: 20970851.
2. Fortin D. *The blood-brain barrier: its influence in the treatment of brain tumors metastases.* Curr Cancer Drug Targets. 2012;12(3):247–59. PubMed PMID: 22229251.
3. Fu B.M. *Experimental methods and transport models for drug delivery across the blood-brain barrier.* Curr Pharm Biotechnol. 2012;13(7):1346–59. PubMed PMID: 22201587.
4. Huang R., Ke W., Han L., Liu Y., Shao K., Jiang C., Pei Y. *Lactoferrin-modified nanoparticles could mediate efficient gene delivery to the brain in vivo.* Brain Res Bull. 2010;81(6):600–4. PubMed PMID: 20026388.

5. Huang R.Q., Ke W.L., Qu Y.H., Zhu J.H., Pei Y.Y., Jiang C. *Characterization of lactoferrin receptor in brain endothelial capillary cells and mouse brain.* J Biomed Sci. 2007;14(1):121–8. PubMed PMID: 17048089.
6. Suzuki Y.A., Lopez V., Lonnerdal B. *Mammalian lactoferrin receptors: structure and function.* Cell Mol Life Sci. 2005;62(22):2560–75. PubMed PMID: 16261254.
7. Fillebeen C., Descamps L., Dehouck M.P., Fenart L., Benaissa M., Spik G., Cecchelli R., Pierce A. *Receptor-mediated transcytosis of lactoferrin through the blood-brain barrier.* J Biol Chem. 1999;274(11):7011–7. PubMed PMID: 10066755.
8. Qiao R., Jia Q., Huwel S., Xia R., Liu T., Gao F., Galla H.J., Gao M. *Receptor-mediated delivery of magnetic nanoparticles across the blood-brain barrier.* ACS Nano. 2012;6(4): 3304–10. PubMed PMID: 22443607.