Lactoferrin-conjugated poly(ethylene glycol)-coated Fe_3O_4 nanoparticles

Fe_3O_4-Lf

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Chemical name:	Lactoferrin-conjugated poly(ethylene glycol)-coated Fe_3O_4 nanoparticles	
Abbreviated name:	Fe_3O_4-Lf	
Synonym:		
Agent Category:	Nanoparticles	
Target:	Lactoferrin receptors	
Target Category:	Receptors	
Method of detection:	Magnetic resonance imaging (MRI)	
Source of signal / contrast:	Fe_3O_4	
Activation:	No	
Studies:	• *In vitro* • Rodents	No structure available.

Background

[PubMed]

The lactoferrin (Lf)-conjugated poly(ethylene glycol) (PEG)-coated Fe_3O_4 nanoparticles, abbreviated as Fe_3O_4-Lf, was synthesized by Qiao et al. for use as a contrast agent for magnetic resonance imaging (MRI) of the brain (1). As a ligand, Lf acts to enhance the blood–brain barrier (BBB) penetration and Lf receptor-targeting of Fe_3O_4-Lf.

BBB is composed of tight junction-sealed brain capillary endothelial cells (BCECs) and supporting pericytes and astrocytic endfeet (2). Exogenous compounds are prevented by

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the BBB from reaching the brain by passive transport or 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 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 (1, 7). Studies with membrane preparations of mouse brains 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 (1). Lf receptor has also been shown to be overexpressed in various tumors including brain glioma (8). Because of these features, Lf has been applied as a ligand in designing brain-targeted delivery systems.

Xie et al. conjugated Lf to superparamagnetic iron oxide nanoparticles (SPIONs) to develop the contrast agent Lf-SPION (8), while Qiao et al. developed the agent Fe_3O_4-Lf by conjugating Lf to Fe_3O_4 nanoparticles through PEG (1). For the former agent, Lf was designed to target Lf-SPIONs to tumors expressing the Lf receptor. 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 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 Qiao et al. with Fe_3O_4-Lf.

Related Resource Links:

Nucleotide and protein of lactoferrin

Protein of lactoferrin receptor

Lactoferrin-related clinical trials in ClinicalTrials.gov

Synthesis

[PubMed]

Qiao et al. described the synthesis of Fe_3O_4-Lf in detail (1). Briefly, the PEG-coated Fe_3O_4 nanoparticles were synthesized with a "one-pot" synthetic reaction of $Fe(acac)_3$, oleylamine, and HOOC-PEG-COOH in the diphenyl ether solution. Ether was then used to precipitate and purify the Fe_3O_4 nanocrystal. The final product, PEG-coated Fe_3O_4, was formulated in either Milli-Q water or phosphate-buffered saline.

The average size of PEG-coated Fe_3O_4 nanoparticles was 16.5 ± 1.6 nm in diameter, as measured with transmission electron microscopy. The molar transversal relaxivity was 231 $mM^{-1}s^{-1}$. The organic content accounted for ~16.6% of the particles. A room-temperature magnetization curve confirmed that the PEG-coated Fe_3O_4 nanocrystals were superparamagnetic, and the particles exhibited a saturation magnetization of 66.0 emu/g, corresponding to 79.1 emu/g Fe_3O_4.

The Fe_3O_4-Lf conjugate was prepared with the classic EDC/sulfo-NHS-mediated amidation reaction. The hydrodynamic size of PEG-coated Fe_3O_4 as determined with dynamic light scattering was 43.6 nm, and this value increased to 48.9 nm after Lf conjugation, suggesting that Lf was effectively coupled to the PEG-coated Fe_3O_4 nanoparticles. The polydispersity index of Fe_3O_4-Lf (0.386) was similar to that of PEG-coated Fe_3O_4 (0.348), indicating that particles did not coagulate during the coupling reaction. The Bradford method was adopted to determine the protein content and revealed ~14.4 Lf molecules per Fe_3O_4 nanoparticle on average for the Fe_3O_4-Lf. The chemical yield and purity of Fe_3O_4-Lf were not reported.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Qiao et al. first tested the permeability of Fe_3O_4-Lf using an *in vitro* model of the BBB (1). The model was established with primary porcine BCECs that were cultured on microporous filter membrane inserts within a chamber (9, 10). Transendothelial electrical resistance (TEER) was measured after incubation of BCECs with Fe_3O_4-Lf or PEG-coated Fe_3O_4 at concentrations of 0.04, 0.1, and 0.3 mg Fe/mL, respectively (1). Water was used as a reference for the changes of TEER after nanoparticle exposure. TEER is an indicator of BBB integrity and is usually expressed as measured resistance multiplied by the area of endothelial monolayer ($\Omega\ cm^2$). TEER level is mainly determined by the tight junctions between endothelial cells. For the model used by Qiao et al., the TEER was 1,500–2,000 $\Omega\ cm^2$, typically >700 $\Omega\ cm^2$ after 7 days of culture (1).

At the concentration of 0.04 mg Fe/mL, both Fe_3O_4-Lf and PEG-coated Fe_3O_4 decreased the TEER level during the initial several hours of exposure. The TEER level recovered over time. Water resulted in similar changes of TEER. These results suggested that the initial decrease of TEER was mainly due to the cell response to the environmental change. However, compared to water, Fe_3O_4-Lf led to a lower degree of TEER reduction, while PEG-coated Fe_3O_4 resulted in a higher degree of TEER reduction. This trend was more evident at the concentration of 0.1 mg Fe/mL, indicating that the BBB remained more intact in the presence of Fe_3O_4-Lf than PEG-coated Fe_3O_4. At the high concentration of 0.3 mg Fe/mL, both Fe_3O_4-Lf and PEG-coated Fe_3O_4 led to much weaker TEER signals than water, suggesting considerable damage to the tight junctions by both Fe_3O_4-Lf and PEG-coated Fe_3O_4 at this concentration. Overall, the results indicated that Lf could protect the tight junctions from being damaged by the Fe_3O_4 nanoparticles at low

concentrations. Experiments performed by incubating Lf alone with the BCECs further confirmed that Lf could increase the TEER value.

Qiao et al. then evaluated the efficiency of particle transport by measuring the iron content in the media of the basolateral side after ~18 h incubation of the BCECs with either Fe_3O_4-Lf or PEG-coated Fe_3O_4 particles (1). At concentrations of 0.04 and 0.1 mg Fe/mL of the agents at the apical side, the transport efficiency of Fe_3O_4 particles was strongly enhanced by the Lf conjugation (Table 1). The transport of Fe_3O_4-Lf (0.1 mg Fe/mL) could be blocked with Lf, showing that the iron content dropped from 22.0 ± 2.9% to 1.0 ± 0.6% in the presence of 16 times the amount of Lf in the apical media. Consistent with other data, these results also indicate that Lf could facilitate the cross of Fe_3O_4 particles through the BBB.

Table 1 Transport efficiency of Fe_3O_4-Lf or PEG-coated Fe_3O_4 particles in *in vitro* BBB model.

Agents	Initial Fe concentration at apical side (mg/mL)	Fe content at basolateral side (mg/mL)	Transport efficiency (%)[a]
PEG-Fe_3O_4	0.04	0.0045 ± 0.0003	22.5 ± 1.4
Fe_3O_4-Lf		0.0094 ± 0.0028	47.0 ± 13.8
PEG-Fe_3O_4	0.10	0.0048 ± 0.0006	9.6 ± 1.3
Fe_3O_4-Lf		0.0110 ± 0.0015	22.0 ± 2.9

[a]The efficiency was calculated by dividing the feeding amount of Fe by the Fe content in the basolateral medium.

Animal Studies

Rodents

[PubMed]

Qiao et al. evaluated the BBB-penetrating efficiency of Fe_3O_4-Lf *in vivo* with T2*-weighted MRI after intravenous injection of PEG-coated Fe_3O_4 or Fe_3O_4-Lf (10 mg Fe/kg) into Sprague-Dawley rats (n = 2/group) (1). At 15 min after injection, stronger contrast-enhanced vascular images of the brain were obtained with Fe_3O_4-Lf than with PEG-coated Fe_3O_4. Similarly, a stronger effect on reducing T2* value in the thalamus, brain stem, and frontal cortex was observed at 24 h with Fe_3O_4-Lf than with PEG-coated Fe_3O_4. These results supported the conclusion that the BBB-penetrating efficiency of Fe_3O_4 particles could be enhanced through Lf-receptor-mediated transport *in vivo*.

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.

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