H18/7 F(ab')$_2$ E-selectin monoclonal antibody conjugated to cross-linked iron oxide nanoparticles

CLIO-H18/7 F(ab')$_2$

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Chemical name:	H18/7 F(ab')$_2$ E-selectin monoclonal antibody conjugated to cross-linked iron oxide nanoparticles	
Abbreviated name:	CLIO-H18/7 F(ab')$_2$	
Synonym:		
Agent category:	Antibody	
Target:	E-selectin	
Target category:	Receptor	
Method of detection:	Magnetic resonance imaging (MRI)	
Source of signal:	Iron oxide	
Activation:	No	
Studies:	• *In vitro* • Rodents	Click on protein, nucleotide (RefSeq), and gene for more information about E-selectin.

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. Water comprises ~80% of most soft tissue. The contrast of proton MRI depends mainly on the density of nuclear proton spins, the relaxation times

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of the nuclear magnetization (T1, longitudinal and T2, transverse), the magnetic environment of the tissues, and the blood flow to the tissues. However, insufficient contrast between normal and diseased tissues requires development of contrast agents. Most contrast agents affect the T1 and T2 relaxation of the surrounding nuclei, mainly the protons of water. T2* is the spin-spin relaxation time composed of variations from molecular interactions and intrinsic magnetic heterogeneities of tissues in the magnetic field (1).

The superparamagnetic iron oxide (SPIO) structure is composed of ferric iron (Fe^{3+}) and ferrous iron (Fe^{2+}). The iron oxide particles are coated with a protective layer of dextran or other polysaccharide. These particles have large combined magnetic moments or spins, which are randomly rotated in the absence of an applied magnetic field. SPIO is used mainly as a T2 contrast agent in MRI, though it can shorten both T1 and T2/T2* relaxation processes. SPIO particle uptake into the reticuloendothelial system (RES) occurs by endocytosis or phagocytosis. SPIO particles are taken up by phagocytic cells such as monocytes, macrophages, and oligodendroglial cells. A variety of cells can also be labeled with these particles for cell trafficking and tumor-specific imaging studies. SPIO agents are classified by their sizes with coating material (~20–3,500 nm in diameter) as large SPIO (LSPIO) nanoparticles, standard SPIO (SSPIO) nanoparticles, ultrasmall SPIO (USPIO) nanoparticles, and cross-linked iron oxide (CLIO) nanoparticles (1).

USPIO nanoparticles are composed of iron particles of ~4–6 nm diameters and the hydrodynamic diameters are ~20–40 nm. The crystals are covered with a layer of dextran with significant T2 relaxation effects. USPIO nanoparticles have a long plasma half-life as a result of improved coating and small size. In humans, the blood pool half-life of plasma relaxation times is calculated to be >24 h (2) and 1-2 h in mice. Because of its long blood half-life, USPIO nanoparticles can be used as a blood pool agent during the early phase of intravenous administration (3). In the late phase, USPIO nanoparticles are suitable for the evaluation of RES in the body, particularly in lymph nodes (4).

E-selectin is found on the cell surface of endothelial cells (5, 6). It binds to sialy-$Lewis^x$ (a carbohydrate moiety) on the cell-surface of leukocytes. Tumor necrosis factor α (TNFα) and interleukin-1 (IL-1), released from inflammatory stimuli, upregulated E-selectin and other adhesion molecule expression on the vascular endothelial cells, which leads to leukocyte adhesion to the activated endothelium. E-selectin and other selectins are involved in arresting leukocytes on the endothelium. An anti-human E-selectin monoclonal antibody fragment, H18/7 $F(ab')_2$, was conjugated to CLIO nanoparticles for noninvasive MRI of E-selectin expression in endothelial cells (7, 8).

Synthesis

[PubMed]

CLIO nanoparticles were first activated with *N*-succinimidyl 3-(2-pyridyldithio)propionate (SPDP) (7). CLIO nanoparticles bearing 2-pyridyldithiopropiony moieties were conjugated with H18/7 F(ab')2 fragments by using

N-succinimidyl *S*-acetylthioacetate (SATA) as a coupling reagent to form CLIO-H18/7 F(ab')$_2$. CLIO-H18/7 F(ab')$_2$ had a hydrodynamic diameter of 45 ± 2.5 nm with five molecules of H18/7 F(ab')$_2$. CLIO-H18/7 F(ab')$_2$ and CLIO exhibited R2 values of 48 and 55 mM^{-1} s^{-1} at 0.47T and 40°C, respectively.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Kang et al. (7) performed *in vitro* binding studies with the use of cultured human umbilical vein endothelial cells (HUVECs) in the presence or absence of IL-1β. IL-1β–activated HUVECs accumulated 83–104 ng iron/10^6 cells after incubation with 1.2–2.4 μg iron/ml of CLIO-H18/7 F(ab')$_2$ for 60 min. Unactivated HUVECs accumulated only 0.05–0.1 ng iron/10^6 cells. Therefore, HUVECs treated with IL-1β expressed 100–200 times more binding of E-selectin to CLIO-H18/7 F(ab')$_2$ than control HUVECs. IL-1β–activated HUVECs (T2, 29–40 ms) showed a stronger MRI contrast enhancement than the control HUVECs (T2, 1,465–1,500 ms). There was no specific binding to IL-1β–activated HUVECs when incubated with CLIO or CLIO conjugated with a nonspecific antibody F(ab')$_2$ fragment. The binding of CLIO-H18/7 F(ab')$_2$ to IL-1β–activated HUVECs was inhibited by 76% with a 25-fold molar excess of H18/7 F(ab')$_2$.

Animal Studies

Rodents

[PubMed]

Kang et al. (8) performed *in vivo* imaging of E-selectin expression in functional blood vessels by implanting HUVECs in Matrigel in athymic mice. The formation of HUVEC-containing vessels was established by histology and microscopy. CLIO-F(ab')$_2$ probes were injected after the IL-1β–induced E-selectin expression. High-resolution (7T) MRI images were obtained before and after the administration of CLIO-H18/7 F(ab')$_2$, which showed a specific intensity decrease only if treated with IL-1β. A three-fold higher CLIO-induced MRI signal decrease on T2* images was measured in HUVEC implants in response to IL-1β treatment. MRI signal intensity did not change in control animals that: (a) harbored Matrigel alone, (b) were not given IL-1β treatment, or (C) were injected with CLIO linked to control F(ab')$_2$. Analyses by histology and transmission electron microscopy of the Matrigel implants revealed that the majority of detectable iron oxide was localized inside the lumina of neovessels induced by IL-1β and 25% of total cell-associated particles were accumulated in HUVECs.

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.

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