Anti-ligand–induced binding sites (LIBS) antibody conjugated to microparticles of iron oxide

LIBS-MPIOs

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Chemical name:	Anti-ligand–induced binding sites (LIBS) antibody conjugated to microparticles of iron oxide	
Abbreviated name:	LIBS-MPIOs	
Synonym:		
Agent category:	Antibody, single-chain	
Target:	Platelet glycoprotein GPIIb/IIIa receptor (CD61/CD41)	
Target category:	Receptor	
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 density of nuclear proton spins, the relaxation

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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 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). Cross-linked iron oxide (CLIO) 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 increased contrast images.

Thrombosis plays a major role in many cardiovascular diseases, such as myocardial infarction, pulmonary embolism (PE), deep venous thrombosis (DVT), atherothrombosis, or cerebral venous thrombosis (2, 3). DVT is a significant cause of PE, which is a potentially life-threatening clinical problem. Thrombosis occurs when platelets deposit in regions of low flow in the deep venous system, followed by an activation process of thrombin, which then converts fibrinogen into fibrin. Platelets become activated and bind to fibrinogen, resulting in platelet aggregation *via* the platelet integrin GPIIb/IIIa ($\alpha_{IIb}\beta_3$, CD61/CD41). The thrombus may become organized or detached from the vessel wall. A single-chain antibody (anti-LIBS 145) has been developed to recognize ligand-induced binding sites (LIBS) of GPIIb/IIIa that become exposed only upon receptor-ligand binding (4). Anti-LIBS 145 single-chain antibody does not bind to circulating platelets. Anti-LIBS 145 single-chain antibody was conjugated to microparticles of iron oxide (MPIOs) to form LIBS-MPIOs for T2-weighted MRI imaging of platelet-containing thrombi (5-8).

Related Resource Links:

- Chapters in MICAD
- Gene information in NCBI (GPIIIa/CD61, GPIIb/CD41)
- Articles in OMIM (GPIIIa/CD61, GPIIb/CD41)
- Clinical trials (Integrin)
- Drug information in FDA

Synthesis

[PubMed]

Cobalt-functionalized MPIOs (1 mg, 1 μm in diameter) were incubated with LIBS containing histidine tag for 10 min at room temperature (8). LIBS-MPIOs were isolated with magnetic separation and washings. Approximately 10 μg LIBS antibody was conjugated to 1 mg MPIOs. An irrelevant single-chain control antibody was also conjugated to MPIOs. LIBS-MPIOs exhibited r_1 and r_2 relaxivity values of 0.05 ± 0.01 and 121.2 ± 6.8 $mM^{-1}s^{-1}$ (8), respectively.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Confocal microscopy of human platelets immobilized on fibrinogen showed that LIBS-MPIOs bound to platelets co-localizing with anti-CD61 antibody (5). MPIOs conjugated with control antibody showed little binding to the platelets.

Animal Studies

Rodents

[PubMed]

von zur Muhlen et al. (5) performed *ex vivo* T2-weighted MRI (11.7 T) of injured arterial segments perfused with 6.5 mg iron/kg LIBS-MPIOs ($n = 7$) or control MPIOs ($n = 6$). MRI signal decreases in the thrombosed artery were 23.7 and 6.2 for LIBS-MPIOs and control MPIOs ($P < 0.01$), respectively. There was a significantly higher binding ($P < 0.01$) for LIBS-MPIOs (9.98 LIBS-MPIOs/section) than control MPIOs (0.5 control MPIOs/section). Immunostaining with anti-CD61 confirmed the co-localization of LIBS-MPIOs and platelets adherent to the thrombosed arterial wall. An *ex vivo* biodistribution study was performed in mice ($n = 3$) at 0.5 h and 24 h after intravenous injection of 4.5 mg iron/kg. At 30 min, there were 7, 2, 32, and 110 LIBS-MPIOs/section for the kidney, lung, liver, and spleen, respectively. At 24 h, there were 2, 12, 28, and 100 LIBS-MPIOs/section for the kidney, lung, liver, and spleen, respectively. The high uptake in the liver and spleen represented sequestration of the nanoparticles by the phagocytes present in these organs. No blocking experiment was performed.

von zur Muhlen et al. (8) performed *in vivo* MRI (9.4 T) of thrombosis and thrombolysis in mice ($n = 6$–8 mice/group) exposed to 6% ferric chloride solution to injure the carotid artery. A significant signal decrease in the thrombosed artery was observed with LIBS-MPIOs but not with control MPIOs at 36 min after injection ($P < 0.05$). At 48 min after injection of LIBS-MPIOs, human or murine urokinase was injected to induce thrombolysis. Both urokinases reduced the signal decreases at 24–36 min after injection ($P < 0.05$). Histological examination of the arterial wall showed that there were 30, 3, and 4 LIBS-MPIOs/thrombus section for untreated, human urokinase-treated, and murine urokinase-treated mice. There was a significant correlation of the MPIO content with MRI signal voids ($P < 0.01$).

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.

References

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