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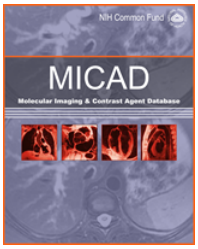

Anti-vascular cell adhesion molecule antibody M/K-2.7 and anti-P-selectin antibody RB40.34 conjugated microparticles of iron oxide

VCAM-MPIO-P-selectin

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Chemical name:	Anti-vascular cell adhesion molecule antibody M/K-2.7 and anti-P-selectin antibody RB40.34 conjugated microparticles of iron oxide	
Abbreviated name:	VCAM-MPIO-P-selectin	
Synonym:	M/K-2.7-MPIO-RB40.34	
Agent Category:	Antibody	
Target:	Vascular cell adhesion molecule-1 (VCAM-1) and P-selectin	
Target Category:	Antibody-antigen binding	
Method of detection:	Magnetic resonance imaging (MRI)	
Source of signal/contrast:	Iron oxide	
Activation:	No	
Studies:	• *In vitro* • Rodents	Click on protein, nucleotide (RefSeq), and gene for more information about VCAM-1. Click on protein, nucleotide (RefSeq), and gene for more information about P-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, 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 (T_1, longitudinal; T_2, 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 T_1 and T_2 relaxation times of

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the surrounding nuclei, mainly the protons of water. T_2^* 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) nanoparticles and other iron oxide formulations affect T_2 primarily and lead to decreased signals. On the other hand, paramagnetic T_1 agents such as gadolinium (Gd^{3+}) and manganese (Mn^{2+}) accelerate T_1 relaxation and lead to brighter contrast images.

Endothelial cells are important cells in inflammatory responses (2, 3). Bacterial lipopolysaccharide (LPS), virus, inflammation, and tissue injury increase tumor necrosis factor α (TNFα), interleukin-1 (IL-1), and other cytokine and chemokine secretion. Emigration of leukocytes from blood is dependent on their ability to roll along endothelial cell surfaces and subsequently 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 (VCAM-1), integrins, and selectins. Chemokines are chemotactic toward leukocytes and toward sites of inflammation and tissue injury. The movements of leukocytes through endothelial junctions into the extravascular space are highly orchestrated through various interactions with different adhesion molecules on endothelial cells (4).

P-selectin is found on the cell surface of endothelial cells and platelets (3, 5). It binds to glycoproteins on the cell surface of leukocytes. P-selectin and other selectins are involved in rolling and arresting leukocytes on the endothelium. VCAM-1 is found in very low levels on the cell surface of resting endothelial cells and other vascular cells, such as smooth muscle cells and fibroblasts (6-10). VCAM-1 binds to its counterligand, very late antigen-4 (VLA-4) integrin, on the cell surface of leukocytes. IL-1 and TNFα increase expression of VCAM-1, P-selectin, and other cell adhesion molecules on the vascular endothelial cells, which leads to leukocyte adhesion to the activated endothelium. Furthermore, VCAM-1 expression was also induced by oxidized low-density lipoproteins under atherogenic conditions (11). Overexpression of VCAM-1 by atherosclerotic lesions plays an important role in their progression to vulnerable plaques, which may erode and rupture. Microparticles of iron oxide (MPIOs) are composed of iron particles with diameters of ~4.5 μm. MPIO targeted with anti-VCAM-1 monoclonal antibody (mAb) M/K-2.7 and anti-P-selectin mAb RB40.34 (VCAM-MPIO-P-selectin) is being developed as a non-invasive, dual-targeted agent for VCAM-1 and P-selectin expression in vascular endothelial cells during different stages of inflammation in atherosclerosis (12).

Synthesis

[PubMed]

The synthesis of VCAM-MPIO-P-selectin was described by McAteer et al. (12). The p-toluenesulphonyl-MPIOs were commercially available (Invitrogen). M/K-2.7 and RB40.34 mAbs (2.5 μg of each/10^7 MPIO) were incubated with the activated MPIO at 37°C for 20 h. Single-targeted VCAM-MPIO or P-selectin-MPIO and IgG control MPIO (IgG-MPIO) were prepared in the same way with 5 μg of each mAb. MPIOs were washed and incubated with 0.1% BSA at 37°C for 4 h to block the remaining activated sites.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

McAteer et al. (12) performed *in vitro* cell-binding assays with VCAM-MPIO using mouse sEND-1 endothelial cells. Using light microscopy, VCAM-MPIO bound to only TNF-activated sEND-1 cells ($R_2 = 0.94$, $P = 0.03$) in a dose-dependent manner, whereas IgG-MPIO did not. A corresponding decrease in MRI signal intensity of MPIO was observed MRI images at 11.7 T ($R_2 = 0.98$, $P = 0.01$), indicating the presence of VCAM-MPIO on the cell surface. VCAM-MPIO binding was blocked by pre-incubation with soluble VCAM-1 and not with ICAM-1. Unconjugated MPIO were phagocytosed by mouse peritoneal macrophages and not by sEND-1 cells.

Animal Studies

Rodents

[PubMed]

VCAM-MPIO-P-selectin (6×10^7 MPIO, 30 mg iron/kg) was administered in apolipoprotein $E^{-/-}$ mice ($n = 6$) (12). Using light microscopy, the number of dual-targeted MPIOs that bound to endothelium overlying aortic root atherosclerosis was 5.9-fold more than P-selectin-MPIOs (16.1 ± 2.7 per section) ($n = 4$) ($P < 0.05$) and 4.5-fold more than VCAM-MPIOs (12.9 ± 4.2) ($n = 7$) ($P < 0.01$). The number of IgG_1-MPIOs that bound was 1.7 ± 4.2. VCAM-MPIO-P-selectin was injected intravenously in apolipoprotein $E^{-/-}$ mice *via* the jugular vein ($n = 4$). The number of VCAM-MPIO-P-selectin that bound aortic root endothelium was quantifiable with *ex vivo* MRI (2.5-fold increase *versus* IgG_1-MPIO (14 ± 4.2 *versus* 4 ± 1.8); $P < 0.01$). The MPIOs were well tolerated in apolipoprotein $E^{-/-}$ mice, and histological examination showed that the lungs had high accumulation at 30 min after injection with redistribution to the spleen and liver at 24 h. No blocking experiments were performed.

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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