VCAM-1 internalizing peptide-28 nanoparticles

VINP-28 NP

Kam Leung, PhD[1]

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Chemical name:	VCAM-1 internalizing peptide-28 nanoparticles	
Abbreviated name:	VINP-28 NP	
Synonym:	VHPKQHRGGSK(FITC)GC-CLIO-Cy5.5 nanoparticles, VINP-28-CLIO-Cy5.5 nanoparticles	
Agent Category:	Peptide	
Target:	Vascular cell adhesion molecule-1 (VCAM-1)	
Target Category:	Receptor binding	
Method of detection:	Magnetic resonance imaging (MRI), optical (near-infrared (NIR) fluorescence)	
Source of signal/ contrast:	Iron oxide, Cy5.5	
Activation:	No	
Studies:	• *In vitro* • Rodents	Click on protein, nucleotide (RefSeq), and gene for more information about VCAM-1.

Background

[PubMed]

Optical fluorescence imaging is increasingly being used to obtain images of biological functions of specific targets *in vitro* and in small animals (1, 2). Near-infrared (NIR) fluorescence (700–900 nm) detection avoids the background fluorescence interference of natural biomolecules, providing a high contrast between target and background tissues. NIR fluorescence imaging is becoming a non-invasive alternative to radionuclide imaging *in vitro* and in small animals.

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

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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 nuclei (proton spins), the relaxation 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 times 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 (3). Cross-linked iron oxide (CLIO) nanoparticles and other iron oxide formulations affect T2 primarily and lead to a decreased signal.

A multimodal nanoparticle probe that consists of a contrast agent and a NIR fluorochrome may provide consistent information. CLIO nanoparticles can be internalized by cells of the reticuloendothelial system and have long circulating times within an animal body. The blood half-life of CLIO is ~10 h in mice (4). The accumulation of nanoparticles in cells causes a reduction in signal intensity with T2-weighted (T2*W) spin-echo pulse sequences. NIR fluorochromes (e.g., Cy5.5) provide an improved optical (NIR) signal from tissue. CLIO-Cy5.5 has been developed as a multimodal probe for imaging.

Endothelial cells are important in inflammatory responses (5, 6). Bacterial lipopolysaccharide, 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 (ICAM-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 movement of leukocytes through endothelial junctions into the extravascular space is highly orchestrated through various interactions with different adhesion molecules on endothelial cells (7).

VCAM-1 is found in very low amounts on the cell surface of resting endothelial cells and other vascular cells, such as smooth muscle cells and fibroblasts (8-12). VCAM-1 binds to very late antigen-4 (VLA-4) integrin on the cell surface of leukocytes. IL-1 and TNFα increase expression of VCAM-1 and other cell adhesion molecules on the vascular endothelial cells, which leads to leukocyte adhesion to the activated endothelium. Furthermore, VCAM-1 expression is also induced by oxidized low-density lipoproteins under atherogenic conditions (13). Overexpression of VCAM-1 by atherosclerotic lesions plays an important role in their progression toward vulnerable plaques, which may erode and rupture. CLIO nanoparticle targeted with anti–VCAM-1 antibody is being developed as a non-invasive agent for VCAM-1 expression in vascular endothelial cells during

different stages of inflammation in atherosclerosis (14). A linear peptide (VHPKQHR) homologous to VLA-4 bound to and was internalized by cells expressing VCAM-1 (15, 16). VHPKQHRGGSK(FITC)GC (VINP-28) with fluorescein isothiocyanate (FITC) was synthesized and conjugated to CLIO-Cy5.5 nanoparticle to form a multimodal imaging agent (VINP-28-CLIO-Cy5.5) that consists of CLIO nanoparticle with attachment of VINP-28 and Cy5.5.

Synthesis

[PubMed]

The synthesis of VINP-28-CLIO-Cy5.5 nanoparticles was described by Kelly et al. (15) and Nahrendorf et al. (16). The amino-CLIO nanoparticles (62 amino groups per nanoparticle, 3 nm Fe core, 38 nm in diameter with dextran coating, R2 = 62 $mM^{-1}s^{-1}$) were labeled with Cy5.5 using *N*-hydroxysuccinimide to form amino-CLIO-Cy5.5 nanoparticles, which were then derivatized with succinimidyl iodoacetate and conjugated with VINP-28 *via* the cysteine residue in the linker region. The purified VINP-28-CLIO-Cy5.5 nanoparticles had 12 VINP-28 and 2 Cy5.5 molecules per nanoparticle.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Kelly et al. (15) performed cell-binding assays with VINP-28 using murine cardiac endothelial cells with a binding affinity of 33.7 ± 9 nM as determined with fluorescence flow cytometry. The peptide accumulated intracellularly, and the accumulation was significantly inhibited ($P < 0.001$) by 75% with soluble VCAM-1 but not with ICAM-1. VINP-28-CLIO-Cy5.5 nanoparticles exhibited a 3.2-fold increased in fluorescent signal over the VINP-28. VINP-28-CLIO-Cy5.5 bound to endothelial cells with 9-fold and 5-fold increase in fluorescent signal over macrophages and fibroblasts, respectively. *In vitro* MRI studies with VINP-28-CLIO-Cy5.5 nanoparticles in human carotid endarterectomy atheroma specimens showed a T2 reduction of 3.8 ± 1.7 ms ($P < 0.05$ *versus* saline), which is consistent with fluorescence imaging and immunohistochemistry and indicates the presence of VCAM-1 expression cells in the atheroma plaque.

Animal Studies

Rodents

[PubMed]

Nahrendorf et al. (16) performed intravital confocal microscopy in C57BL/6 mice after subcutaneous injection of TNFα to the right ear, resulting in vascular inflammation in 24 h. After injection of VINP-28-CLIO-Cy5.5, there was a strong enhancement in endothelial cells expressing VCAM-1 in the inflamed ear. In normal mice, VINP-28-CLIO-Cy5.5 exhibited a blood half-life of 17.7 h. The organ with the highest accumulation

(injected dose/g) at 6 h after injection was the liver (2.83%), followed by the kidneys (1.87%), heart (0.90%), spleen (0.82%), lungs (0.77%), muscle (0.22%), and fat (0.20%).

Nahrendorf et al. (16) performed *in vivo* MRI and optical imaging in apolipoprotein E–deficient (apoE$^{-/-}$) mice ($n = 28$) after injection with VINP-28-CLIO-Cy5.5 or saline. There was a 77% ($P < 0.05$) decrease in MR signal intensity in the aortic wall 48 h after injection. The aortic arch exhibited a high plaque/background ratio of 9.25 ± 2.5 (>350% higher than saline control), revealing signal enhancement in the aortic arch of mice that received VINP-28-CLIO-Cy5.5 ($P < 0.05$). VINP-28-CLIO-Cy5.5 fluorescence signal colocalized with endothelial cells and macrophages that expressed VCAM-1 and was spatially distinct compared with untargeted control nanoparticles. Atheromata of atorvastatin-treated apoE$^{-/-}$ mice on a high-cholesterol diet showed reduced VINP-28-CLIO-Cy5.5 deposition and VCAM-1 expression as compared with untreated mice ($P < 0.05$). No blocking experiment was 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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References

1. Achilefu S. Lighting up tumors with receptor-specific optical molecular probes. Technol Cancer Res Treat. 2004;**3**(4):393–409. PubMed PMID: 15270591.
2. Ntziachristos V., Bremer C., Weissleder R. Fluorescence imaging with near-infrared light: new technological advances that enable in vivo molecular imaging. Eur Radiol. 2003;**13**(1):195–208. PubMed PMID: 12541130.
3. Wang Y.X., Hussain S.M., Krestin G.P. Superparamagnetic iron oxide contrast agents: physicochemical characteristics and applications in MR imaging. Eur Radiol. 2001;**11**(11):2319–31. PubMed PMID: 11702180.

4. Wunderbaldinger P., Josephson L., Bremer C., Moore A., Weissleder R. Detection of lymph node metastases by contrast-enhanced MRI in an experimental model. Magn Reson Med. 2002;**47**(2):292–7. PubMed PMID: 11810672.
5. Cybulsky M.I., Gimbrone M.A. Endothelial expression of a mononuclear leukocyte adhesion molecule during atherogenesis. Science. 1991;**251**(4995):788–91. PubMed PMID: 1990440.
6. Lowe J.B. Glycosylation in the control of selectin counter-receptor structure and function. Immunol Rev. 2002;**186**:19–36. PubMed PMID: 12234359.
7. Vanderslice P., Woodside D.G. Integrin antagonists as therapeutics for inflammatory diseases. Expert Opin Investig Drugs. 2006;**15**(10):1235–55. PubMed PMID: 16989599.
8. Bochner B.S., Luscinskas F.W., Gimbrone M.A., Newman W., Sterbinsky S.A., Derse-Anthony C.P., Klunk D., Schleimer R.P. Adhesion of human basophils, eosinophils, and neutrophils to interleukin 1-activated human vascular endothelial cells: contributions of endothelial cell adhesion molecules. J Exp Med. 1991;**173**(6):1553–7. PubMed PMID: 1709678.
9. Kume N., Cybulsky M.I., Gimbrone M.A. Lysophosphatidylcholine, a component of atherogenic lipoproteins, induces mononuclear leukocyte adhesion molecules in cultured human and rabbit arterial endothelial cells. J Clin Invest. 1992;**90**(3):1138–44. PubMed PMID: 1381720.
10. Leung K.H. Release of soluble ICAM-1 from human lung fibroblasts, aortic smooth muscle cells, dermal microvascular endothelial cells, bronchial epithelial cells, and keratinocytes. Biochem Biophys Res Commun. 1999;**260**(3):734–9. PubMed PMID: 10403835.
11. Luscinskas F.W., Cybulsky M.I., Kiely J.M., Peckins C.S., Davis V.M., Gimbrone M.A. Cytokine-activated human endothelial monolayers support enhanced neutrophil transmigration via a mechanism involving both endothelial-leukocyte adhesion molecule-1 and intercellular adhesion molecule-1. J Immunol. 1991;**146**(5):1617–25. PubMed PMID: 1704400.
12. Nagel T., Resnick N., Atkinson W.J., Dewey C.F., Gimbrone M.A. Shear stress selectively upregulates intercellular adhesion molecule-1 expression in cultured human vascular endothelial cells. J Clin Invest. 1994;**94**(2):885–91. PubMed PMID: 7518844.
13. Aikawa M., Libby P. The vulnerable atherosclerotic plaque: pathogenesis and therapeutic approach. Cardiovasc Pathol. 2004;**13**(3):125–38. PubMed PMID: 15081469.
14. Tsourkas A., Shinde-Patil V.R., Kelly K.A., Patel P., Wolley A., Allport J.R., Weissleder R. In vivo imaging of activated endothelium using an anti-VCAM-1 magnetooptical probe. Bioconjug Chem. 2005;**16**(3):576–81. PubMed PMID: 15898724.
15. Kelly K.A., Nahrendorf M., Yu A.M., Reynolds F., Weissleder R. and In Vivo Phage Display Selection Yields Atherosclerotic Plaque Targeted Peptides for Imaging. Mol Imaging Biol. 2006.
16. Nahrendorf M., Jaffer F.A., Kelly K.A., Sosnovik D.E., Aikawa E., Libby P., Weissleder R. Noninvasive vascular cell adhesion molecule-1 imaging identifies inflammatory

activation of cells in atherosclerosis. Circulation. 2006;**114**(14):1504–11. PubMed PMID: 17000904.