Cross-linked iron oxide–C-AHA-AREPPTRTFAYWGK(FITC)

CLIO-EPPT

The MICAD Research Team

Created: July 26, 2006; Updated: August 14, 2006.

Chemical name:	Cross-linked iron oxide–C-AHA-AREPPTRTFAYWGK(FITC)	
Abbreviated name:	CLIO-EPPT	
Synonym:		
Agent Category:	Iron oxide, peptide	
Target:	Tumor antigen	
Target Category:	Binding	
Method of detection:	Magnetic resonance imaging (MRI), Optical (NIRF)	
Source of signal:	Iron oxide, Cy5.5, FITC	
Activation:	No	
Studies:	• *In vitro* • Rodents	Click on the above structure for additional information in PubChem.

Background

[PubMed]

Early tumor detection is crucial for successful cancer therapy and can greatly improve a patient's survival rate. Equally important is the *in vivo* assessment of the outcome of cancer therapy. However, developing effective methods to serve these purposes remains one of the most difficult challenges. It would be very useful to develop a method that can non-invasively detect tumors at an early stage and also can monitor tumor progression/regression during the course of therapy.

NLM Citation: The MICAD Research Team. Cross-linked iron oxide–C-AHA-AREPPTRTFAYWGK(FITC). 2006 Jul 26 [Updated 2006 Aug 14]. In: Molecular Imaging and Contrast Agent Database (MICAD) [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2004-2013.

An agent that specifically targets tumors with the combination of high-resolution imaging modalities would allow one to extract anatomical, physiological, and molecular information from the field of interest. Magnetic resonance (MR) imaging provides high-resolution spatial images in small animals and in humans (1). Near-infrared fluorescence (NIRF) imaging has less interference from tissue than short-wavelength fluorescence and provides great signal-to-noise ratio in small animals. Therefore, the combination of MR and NIRF technologies into an agent that possesses a tumor-specific function would allow one to obtain comprehensive information about tumor location, environment, and status in small animals (2, 3).

Mucin-1 (MUC-1) is a transmembrane molecule that is expressed by most glandular epithelial cells. Underglycosylated mucin-1 antigen (uMUC-1) is one of the early hallmarks of tumorigenesis and is overexpressed and underglycosylated in almost all human epithelial cell adenocarcinomas and in non-epithelial cancer cell lines, as well as in hematological malignancies (3). MUC-1 is heavily glycosylated in normal tissues, whereas in neoplastic tissues MUC-1 is underglycosylated. The oligosaccharide chains of uMUC-1 are prematurely terminated by the addition of sialic acids, thus permitting the immune system to access the peptide core of this antigen and reveal epitopes. This feature makes it possible to design a probe that discriminates between the normal cells and adenocarcinoma cells. Therefore, uMUC-1 antigen becomes an excellent target for imaging that can be recognized by a probe that exhibits multiple imaging modalities.

A multimodal imaging probe featuring imaging properties (CLIO-Cy5.5, MR, and NIRF) and a short peptide (C-AHA-AREPPTRTFAYWGK(FITC), or EPPT) that specifically recognizes human uMUC-1 antigen was designed. CLIO-Cy5.5 is a multimodal agent that consists of a super-paramagnetic iron oxide nanoparticle (CLIO) and a NIR fluorochrome (Cy5.5). It serves as a contrast agent for MR imaging and at the same time provides a NIRF image. The EPPT peptide was derived from the third heavy-chain complementarity-determining region (CDR3 V_h) of the antibody ASM2 raised against human epithelial cancer cells (4, 5).

Synthesis

[PubMed]

The synthesis of CLIO-EPPT was described by Medarova et al. (2) and Moore et al (3). In brief, EPPT peptides were synthesized using solid-phase peptide synthesis *via* 9-fluorenylmethyloxycarbonyl (Fmoc) chemistry. A 6-aminohexanoic acid (AHA) served as a linker between the EPPT peptide and the terminal cysteine, which was utilized through a thiol group to conjugate the peptide with CLIO nanoparticles. Fluorescein isothiocyanate (FITC) was incorporated into the peptide through the side chain amino group on lysine. CLIO-NH_2 nanoparticles (produced by mixing CLIO nanoparticles with dextran, followed by activation with epichlorohydrin and ammonia) (6, 7), were mixed with Cy5.5 solution and incubated at room temperature overnight. Purified Cy5.5-CLIO-NH_2 was further activated by reacting with succinimidyl iodoacetate. The activated

Cy5.5-CLIO-NH_2 was then conjugated with the EPPT peptide through the thiol group of the terminal cysteine. The size of the nanoparticle was determined using laser light scattering to be 35.8 nm, with an average of 14 EPPT peptides and 5 Cy5.5 dyes per iron oxide particle.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

A variety of human uMUC-1–positive tumor cell lines from different organs were used to quantitate CLIO-EPPT binding ability (3). These cell lines include ZR75-1 (breast), BT-20 (breast), HT-29 (colon), CAPAN-2 (pancreas), LS174T (colon), and ChaGo-K-1 (lung). The control cell lines include an uMUC-1–negative U87 glioblastoma line and normal lines that are heavily glycosylated (MCF10A and embryonic kidney 293 cell line). The control cell lines displayed a significantly lower binding of ^{125}I-labeled CLIO-EPPT compared with the adenocarcinoma cell lines. Similar results were obtained from flow cytometry analysis, although the staining intensities varied, possibly due to varying glycosylation of different cell lines.

Animal Studies

Rodents

[PubMed]

MR and NIRF images of mice injected with bilateral uMUC-1–positive and uMUC-1–negative tumors were taken before and 24 h after the i.v. injection of CLIO-EPPT (3). Significant MR signal reduction was observed in some regions of uMUC-1–positive tumors (52%, 53%, and 43% decrease for LS174T, CAPAN-2, and ChaGo-K-1 tumors, respectively, *versus* a 13–18% decrease in the uMUC-1–negative control U87 tumors). NIRF images taken immediately after the MR scan showed high NIRF intensities from the uMUC-1–positive tumors (HT-29, LS174T, ChaGo-K-1, and CAPAN-2), whereas no significant signal was observed from the uMUC-1–negative control U87 tumor. The data from NIRF imaging showed that uMUC-1–positive tumors accumulated 3.4-fold more CLIO-EPPT than uMUC-1–negative tumors.

A preclinical orthotopic model of human pancreatic cancer was used to demonstrate the tumor-specific accumulation of CLIO-EPPT (2). The uMUC-1–positive human pancreatic adenocarcinoma cell line CAPAN-2 was surgically implanted into mice. CLIO-EPPT was injected intravenously into the mice 13 days after tumor implantation. MR images were taken before and 24 h after the administration of CLIO-EPPT. Both the reduction of T2 relaxation rates and the high NIRF signal at the tumor location indicated the accumulation of CLIO-EPPT on the tumor. Tumor progression/regression was monitored with CLIO-EPPT after chemotherapeutic treatment. MR imaging results showed that there was no change in tumor size of the treated mice, whereas the untreated mice had a three-fold size increase 21 days after the tumor implantation. Semi-

quantitative analysis from NIRF imaging showed 2.8-fold size increases in untreated mice and no changes in treated mice.

Other Non-Primate Mammals

[PubMed]

No relevant publication is currently available.

Non-Human Primates

[PubMed]

No relevant publication is currently available.

Human Studies

[PubMed]

No relevant publication is currently available.

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