Cys-Arg-Glu-Lys-Ala-superparamagnetic iron oxide-Cy7 nanoparticles

CREKA-SPIO-Cy7

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Chemical name:	Cys-Arg-Glu-Lys-Ala-superparamagnetic iron oxide-Cy7 nanoparticles	
Abbreviated name:	CREKA-SPIO-Cy7	
Synonym:		
Agent Category:	Peptide	
Target:	Clotted plasma proteins	
Target Category:	Binding	
Method of detection:	Magnetic resonance imaging (MRI), optical near-infrared (NIR) fluorescence	
Source of signal/contrast:	Iron oxide, Cy7	
Activation:	No	
Studies:	• *In vitro* • Rodents	No structure is available in PubChem.

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

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in vitro and in small animals. 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 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 magnetic resonance imaging (MRI), though it can shorten both T1 and T2/T2* relaxation processes. SPIO particle uptake into the reticuloendothelial system (RES) is by endocytosis or phagocytosis. SPIO particles are also 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 (3).

A multimodal nanoparticle probe that consists of a contrast agent and a NIR fluorochrome may provide consistent imaging information. SPIO is composed of iron nanoparticles that are 4–6 nm diameter with a hydrodynamic diameter with dextran coating of 50 nm. SPIO nanoparticles can be internalized by RES cells and have long circulating times within an animal body. 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 probe for multimodality imaging in small animals (4).

A meshwork of clotted proteins that has been identified in tumor stroma and vessels is absent in normal tissues (5, 6). The tumor-homing peptide Cys-Arg-Glu-Lys-Ala (CREKA) was identified with phage display screening in tumor-bearing mice with minimal binding to normal vessels (7). CREKA was identified as a ligand that bound to the meshwork of clotted proteins in the tumor stroma and blood vessels. CREKA was conjugated to SPIO labeled with Cy7 (CREKA-SPIO-Cy7) to study *in vivo* biodistribution of the nanoparticles in tumor-bearing mice. CREKA-SPIO-Cy7 is a multimodal imaging agent that consists of SPIO nanoparticles (MRI) with attachment of CREKA and Cy7 (NIR).

Synthesis

[PubMed]

Simberg et al. described the synthesis of CREKA-SPIO-Cy7 nanoparticles (7). Cy7 was conjugated to amino-SPIO dextran-coated nanoparticles (~50 nm in diameter) *via* reaction with Cy7-*N*-hydroxysuccinimide, followed by reaction with a bifunctional linker *N*-[*a*-maleimidoacetoxy]succinimide ester and CREKA to form CREKA-SPIO-Cy7 nanoparticles. The multimodal CREKA-SPIO-Cy7 had ~2,000 Cy7 molecules and ~8,000 peptides per nanoparticle. The extinction coefficient of the Cy7 dye is ~100,000 $cm^{-1}M^{-1}$. The quantum yield is ~0.5. The absorption maximum of the Cy7 dye is 740–760 nm, and the emission maximum is 770–790 nm. Fluorescein was used instead of Cy7 for intravital fluorescence microscopy.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Simberg et al. (7) performed clot-binding assays with fluorescein-labeled CREKA-SPIO using murine and human plasma clots. Using fluorescence microscopy, CREKA-SPIO was found to bind to the clots, and CREKA inhibited the binding.

Animal Studies

Rodents

[PubMed]

Simberg et al. (7) performed *in vivo* confocal fluorescence microscopy in nude mice bearing MDA-MB-435 breast cancer xenografts after injection of CREKA-SPIO nanoparticles (1–4 mg/kg Fe). Little accumulation was observed in the tumors, whereas the RES tissues exhibited a high accumulation of CREKA-SPIO nanoparticles at 5–6 h. However, depletion of RES macrophages in the liver with clodronate- or Ni-liposomes prolonged the circulation of CREKA-SPIO five-fold in the blood and greatly increased the accumulation of CREKA-SPIO nanoparticles in the tumor blood vessels as compared with saline pretreatment. Less accumulation was observed in the liver after Ni-liposomal treatment. The iron content was ~six-fold greater in tumor vessels pretreated with Ni-liposomes. Up to 20% of the tumor vessel lumens were filled with fluorescence masses, which also stained positive for fibrin. Furthermore, no co-localization between fibrin and CREKA-SPIO was observed in the liver blood vessels. Little tumor accumulation was observed with control SPIO. Pretreatment with heparin, a strong clotting inhibitor, reduced tumor accumulation of CREKA-SPIO by >50%. Whole-body NIR imaging scans revealed a strong signal enhanced by CREKA-SPIO-Cy7 in the tumors and liver. However, only the tumor signal was inhibited by >50% with heparin pretreatment. MRI and CREKA blocking studies were not 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.

NIH Support

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