Cross-linked iron oxide–transactivator transcription

CLIO-Tat

The MICAD Research Team

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Chemical name:	Cross-linked iron oxide– transactivator transcription	
Abbreviated name:	CLIO-Tat	
Synonym:	Cross-linked superparamagnetic iron oxide–Tat peptide, FITC-CLIO-Tat, CLIO-Tat(FITC), Tat-CLIO	
Agent Category:	Iron oxide	
Target:	Cell membrane	
Target Category:	Adsorptive endocytosis, phagocytosis	
Method of detection:	Magnetic resonance imaging (MRI)	
Source of signal:	Iron oxide	
Activation:	No	
Studies:	• *In vitro* • Rodents	Click on the above structure for additional information in PubChem.

Background

[PubMed]

Investigation of cell migration could lead to understanding such biological events as embryogenesis development, functions of mature cells, vascular remodeling during angiogenesis, and most immune and infectious diseases. Because monitoring cell migration traditionally involves invasive methods such as intravital microscopy or flow cytometry analysis of cells recovered from excised tissue, it has been a long-sought goal to develop non-invasive imaging of cell migration *in vivo*. Because of recent advances in magnetic resonance imaging (MRI), magnetic labeling of cells is one of the techniques

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that can serve this purpose. Cells can be tagged with magnetic particles and then followed *in vivo* using MRI. Two of the common magnetic labeling techniques are: (A) attaching magnetic particles directly to the cell surface or (B) internalizing biocompatible magnetic particles by either endocytosis (fluid-phase or receptor-mediated) or phagocytosis. Surface attachment of magnetic particles can be efficient, but it is usually not applicable for *in vivo* purposes because of rapid reticuloendothelial clearance of these cells. On the other hand, lymphocytes and other cells have been used to internalize nanoparticles through fluid-phase or receptor-mediated endocytosis. However, these methods are generally not very efficient. It is therefore highly desirable to develop more efficient methods for intracellular magnetic labeling.

There are several peptides and proteins with membrane translocation properties that have been described, including penetrin (the third helix homeodomain of Antennapedia) (1), VP22 herpes virus protein (2, 3), anti-DNA monoclonal antibody and a derived peptide (4), and human immunodeficiency virus-1 transactivator transcription (HIV-1 Tat) protein (5). It is hypothesized that using one of the membrane translocation peptides would enable more efficient internalization of the magnetic nanoparticles into cells.

Superparamagnetic iron oxide (IO) has been used in MRI for more than 10 years (6). One of the most representative compounds is the monodisperse IO nanoparticle (MION) (7-9). MION has been used in MR contrast agent research because of its superparamagnetic IO core and is currently in clinical use. MION is coated with dextran to be biocompatible and is further stabilized *via* cross-linking with epichlorohydrin. This cross-linked IO (CLIO) can then be activated by reacting with ammonia, followed by conjugation with membrane translocation peptides.

An HIV-1 Tat peptide that consists of a core of 10 amino acids (amino acids 48-57) has been used to tag the nanoparticles CLIO-NH_2. These nanoparticles are reported to be internalized into cells >100-fold more efficiently than non-modified magnetic particles (10). These CLIO-Tat agents have been used in *in vitro* and *in vivo* studies.

Synthesis

[PubMed]

The synthesis of CLIO-Tat was described by Lewin et al. (11) and Dodd et al (12). CLIO-NH_2 nanoparticles were prepared by coating CLIO nanoparticles with dextran, followed by cross-linking with epichlorohydrin and activation with ammonia (10, 13). Tat peptides were prepared using 9-fluorenylmethyloxycarbonyl (Fmoc) chemistry with incorporation of fluorescein isothiocyanate (FITC) during the peptide synthesis. Two Tat peptides were prepared: GGC*GRKKRRQRRR*K(FITC)-NH_2 and *GRKKRRQRRR*GYK(FITC)C-NH_2 (the italicized amino acids correspond to residues 48-57 of the Tat protein). These peptides were conjugated with CLIO-NH_2 through either N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP) (10, 11) or succinimidyl iodoacetate (SIA) (12, 14). The average number of peptides per CLIO nanoparticle ranges from 4.1 to 11.6 Tat/particle.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

CLIO-GGC*GRKKRRQRRRK*(FITC)-NH_2 (average of 6.7 Tat peptides per CLIO crystal) was used to study the cell-labeling efficiency with murine lymphocytes, human natural killer cells, and HeLa cells (10). With murine lymphocytes, at a loading concentration of 100 μg of Fe/million cells in 1 ml media, there was an uptake of 2,535 ng of Fe/million cells corresponding to 12.7×10^6 IO particles/cell. This was about 100-fold more efficient than CLIO-NH_2 without Tat peptides attached. These internalized particles were located mostly in cytoplasm and nuclear compartments. A similar compound, CLIO-*GRKKRRQRRR*GYK(FITC)C-NH_2 (CLIO-Tat), was used to study the effect on cellular uptake by changing the number of Tat peptides attached to the nanoparticle. The result showed that greater numbers of Tat peptides attached to the nanoparticles facilitated the cellular uptake in a nonlinear fashion (15).

In addition to its magnetic property, CLIO-Tat was tagged with diethylenetriamine pentaacetic acid (DPTA) so that it could be labeled with ^{111}In to confirm the cellular uptake (11). Human hematopoietic $CD34^+$ cells, mouse neural progenitor cells (C17.2), human $CD4^+$ cells, and mouse splenocytes were used to quantitate cellular uptake. Similar labeling efficiencies were observed among these cell types. Up to $0.5\text{-}2 \times 10^7$ nanoparticles/cell were internalized into the cells within 1 hour at 37 °C. Incorporation of the nanoparticles did not affect viability, differentiation, or proliferation of $CD34^+$ cells. T cells loaded with CLIO-*GRKKRRQRRR*GYK(FITC)C-NH_2 showed normal activation and activation-induced cell death (AICD), as well as normal upregulation of CD69, intercellular cell adhesion molecule-1 (ICAM-1) (CD54), L-selectin (CD62L), and Fas (12).

Animal Studies

Rodents

[PubMed]

Cells loaded with radiolabeled CLIO-Tat were used to prove that the labeling of CLIO-Tat would not affect the *in vivo* distribution of labeled cells in mice. Human $CD34^+$ cells were labeled with CLIO-Tat and ^{111}In oxine or ^{111}In oxine alone (as a control) and then injected into nonobese, severe combined immunodeficiency (NOD/SCID) mice (11). The biodistribution determined by ^{111}In showed no difference between the control and the magnetically labeled cells. Approximately 4% injected dose (ID)/g tissue of cells migrated to bone marrow, 15% ID/g tissue were found in spleen, and 22% ID/g tissue in liver. MRI was performed on the bone marrow of NOD/SCID mice 24 h after the injection. The injected human $CD34^+$ cells were detectable at single-cell level and could be recovered by magnetic separation columns.

CLIO-Tat was used to track immune cells in autoimmune diseases (16). Freshly isolated lymphocytes from NOD mice were labeled with CLIO-Tat and then injected into NOD/SCID mice. MRI showed that these CLIO-Tat–labeled cells infiltrated the pancreatic islets of NOD/SCID mice.

T cells isolated from B6 mice were loaded with CLIO-Tat and injected back into the B6 mice through the tail vein (12). There was a 20% reduction of MRI signal intensity in the spleen at 3 h after the injection, which indicated homing of CLIO-Tat–loaded T cells. The intensity remained low even after 24 h.

With 9.7 Tat peptides (*GRKKRRQRRR*GYK(FITC)C-NH_2) attached to the CLIO-NH_2 nanoparticles, the blood half-life was reduced to 47 ± 6 min compared to 655 ± 37 min for the unmodified CLIO-NH_2 (14). The shortened blood half-life of CLIO-Tat is likely caused by the enhanced interaction of CLIO-Tat with phagocytes mediated either by the Tat peptide itself or by enhanced opsonin-mediated phagocytosis. At an injection concentration of 10 mg Fe/kg, CLIO-NH_2 and CLIO-Tat showed similar biodistributions in female Balb C mice. The highest concentration appeared in the liver, spleen, and lymph nodes, ranging from 8.80 to 6.11% ID/g tissue. Results from fluorescence microscopy of the mouse liver showed that CLIO-NH_2 was concentrated in endothelial and Kupffer cells surrounding hepatic blood vessels. However, CLIO-Tat presented as numerous discrete foci throughout the parenchyma 24 h after the intravenous injection. This indicated that CLIO-Tat somehow passed from the vascular compartment and through cells surrounding vessels to achieve the parenchymal distribution. The fluorescence distributed in the liver cells appeared to be similar to that of HeLa cells, where CLIO-Tat showed pronounced nuclear accumulation. It was suggested that, while the nanoparticles determine the organ biodistribution, Tat peptide conjugation to the nanoparticles broadens the intrahepatic distribution to the parenchyma and also significantly reduces the blood half-life.

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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