GKVLAK–(Gadolinium-(1,4,7,10-tetraazacyclododecane-1,4,7-tris(acetic acid *t*-butyl ester)-10-acetic acid monoamide))–GGGGTVQQEL

Gd-DCCP16

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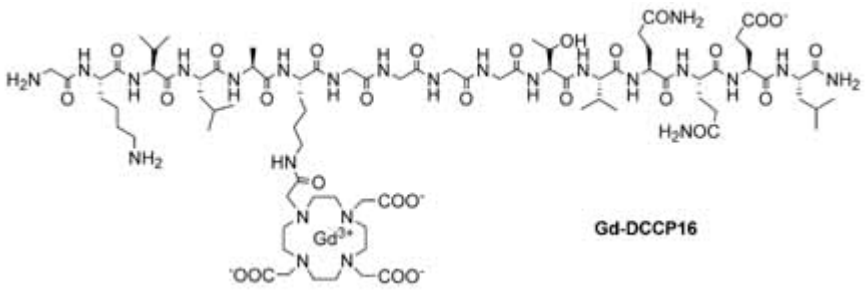
Chemical name:	GKVLAK–(Gadolinium-(1,4,7,10-tetraazacyclododecane-1,4,7-tris(acetic acid *t*-butyl ester)-10-acetic acid monoamide))–GGGGTVQQEL
Abbreviated name:	Gd-DCCP16
Synonym:	
Agent Category:	Peptides
Target:	Transglutaminases
Target Category:	Enzymes
Method of detection:	Magnetic resonance imaging (MRI)
Source of signal / contrast:	Gadolinium (Gd)
Activation:	Yes
Studies:	• *In vitro* • Rodents



Structure of Gd-DCCP16 (1).

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Background

[PubMed]

The gadolinium (Gd)-labeled 16-peptide agent GKVLAK-(Gd-(1,4,7,10-tetraazacyclododecane-1,4,7-tris(acetic acid *t*-butyl ester)-10-acetic acid monoamide (DOTAMA)))-GGGGTVQQEL, abbreviated as Gd-DCCP16, was developed by Tei et al. for magnetic resonance imaging (MRI) of the pathological processes associated with high transglutaminase activity (1).

Transglutaminases are a family of Ca^{2+}-dependent enzymes that catalyze extracellular covalent cross-linking of proteins (2, 3). These enzymes participate in many important biological processes, such as blood coagulation, skin-barrier formation, hardening of the fertilization envelope, and extracellular matrix assembly (2, 4). They are also involved in multiple pathological processes, including wound healing, cancer, myocardial infarctions, and atherothrombosis (5-7). The fibrin-stabilizing factor XIII (FXIII, also known as plasma transglutaminase) and the tissue transglutaminase (TG2) are the two enzymes that have attracted the greatest interest in transglutaminase-targeted imaging and therapy (1, 8, 9). FXIII cross-links fibrin during blood clotting and subsequently produces a mechanically stronger clot with high fibrinolytic resistance, whereas TG2 catalyzes covalent cross-linking of the extracellular matrix. The enzyme-mediated cross-linking is achieved by forming an isopeptide bond between the γ-carbonyl group of a glutamine (Gln) in one protein and the ε-amino group of a lysine (Lys) residue in a nearby protein (3, 9). This cross-link can be blocked by covalent incorporation of an acyl-acceptor amine substrate into the fibrin units or an acyl-donor Gln-containing peptide complementary to the FXIII-reactive Lys donor cross-linking sites of the protein (1). FXIII and TG2 are much more sensitive toward the Gln-bearing substrates than to amine donor Lys residues. These transglutaminase features form the fundamental basis for the development of imaging agents for detection of transglutaminase activity. In recent years, peptides based either on β-casein and α2-antiplasmin for the Gln-donor substrate requirements or on the bovine αA-crystallin for the amine donor substrate requirements have been synthesized for *in vitro* transglutaminase assays, and imaging probes consisting of peptides from α2-antiplasmin have been investigated for mapping the activity of endogenous FXIII and TG2 (10-12).

Tei et al. designed a new model peptide, DCCP16, which was labeled with Gd for MRI and the fluorescent dye IRIS Blue for optical imaging (1). The DCCP16 peptide consists of two moieties. The first moiety is the hexapeptide TVQQEL, which bears two Gln residues

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and one valine residue. The second moiety is the pentapeptide GKVLA, which is known to be a good substrate for transglutaminases. A four-glycine spacer and a Lys residue were inserted between the Lys and the Gln moieties for conjugation of the Gd-DOTAMA or the IRIS Blue-DOTAMA. This spacer-DOTAMA was chosen to keep the Gd- or IRIS Blue-complex far enough from interfering with the active site. The MRI and optical probes were therefore set as GKVLAK-(Gd-DOTAMA)-GGGGTVQQEL (Gd-DCCP16) and GKVLAK-(IRIS Blue-DOTAMA)-GGGGTVQQEL (DCCP16-IRIS Blue), respectively. In vivo effectiveness of the two agents was validated with MRI and optical mapping of transglutaminase-induced agent retention in mouse models of tumor xenografts and blood clotting (1). Noninvasive imaging of transglutaminase activity with Gd-DCCP16 or DCCP16-IRIS Blue provides an important tool for detecting and monitoring transglutaminase-targeted therapy in diverse pathologies including cancer, wound healing, myocardial infarction, and pregnancy failure associated with congenital FXIII deficiency (1). This chapter describes the results generated with Gd-DCCP16. The results obtained with DCCP16-IRIS Blue were described in the chapter on DCCP16-IRIS Blue in MICAD.

Related Resource Links:

- Chapters of FXIII in MICAD
- Articles of transglutaminases in Online Mendelian Inheritance in Man (OMIM)
- Nucleotides and proteins of transglutaminases
- Bioassays of transglutaminase activity in PubChem BioAssay

Synthesis

[PubMed]

The DCCP16 peptide GKVLAKGGGGTVQQEL and the DCCP16 control GAVLAKGGGGTVAAEL were synthesized with solid-phase peptide synthesis with a rink amide resin as solid support and the standard Fmoc strategy (1). As the negative control peptide, the amino acids responsible for the transglutaminase activity of DCCP16 (Gln and Lys) were replaced with alanine. DOTAMA was coupled to the ε-NH_2 group of the Lys residue. The purity values of both GKVLAK-(DOTAMA)-GGGGTVQQEL (DCCP16) and GAVLAK-(DOTAMA)-GGGGTVAAEL (DCCP16 control) were >95%. The yields of DCCP16 and DCCP16 control were 25% (48 mg) and 20% (35 mg), respectively. Chelation with Gd was completed with reactions of $GdCl_3$ and DCCP16 or DCCP16 control. Centrifugation was used to remove $Gd(OH)_3$ from the reaction solution. Orange Xylenol ultraviolet analysis showed that the residual free Gd^{3+} ion was <0.3% (mol/mol) in both Gd-DCCP16 and Gd-DCCP16 control. The relaxivity of Gd-DCCP16 in water was 8.1 $mM^{-1}s^{-1}$ at 0.5 T and 298 K. The relaxivity of Gd-DCCP16 control was not reported.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Two in vitro MRI experiments were performed to determine whether Gd-DCCP16 enters into the clot fibrin network (1). ProHance, a commercially available, nonspecific MRI contrast agent, was selected as the control. Different concentrations of Gd (100–400 M) were tested for both Gd-DCCP16 and ProHance. The first experiment was designed to allow the Gd-DCCP16 to enter the clot formation. Clots were formed in a 2-ml vial with reconstituted human plasma and pathromtin SL. After incubation for two min at 37°C with or without Gd-DCCP16 or ProHance, $CaCl_2$ solution was then added to form the clot. The formed clots were washed to remove the unbound agent and then placed in a 1.5-ml vial for MRI measurements. Notable signal enhancement was observed on clot preparation in which Gd-DCCP16 was part of the clot structure, whereas only a small enhancement was detectable on clots prepared in the presence of ProHance. The second experiment was designed to evaluate the noncovalent interaction between the Gd-DCCP16 and the preformed clot. No evident enhancement was observed when either Gd-DCCP16 or ProHance was incubated with preformed clots. The signal enhancement in MRI images from the first experiment reflected the formation of covalent bonds between the peptide and fibrin, in which Gd-DCCP16 entered into the clot fibrin network as a fibrinogen mimetic. The data in the second experiment suggested the absence of noncovalent interaction between DCCP16 and the preformed clot.

To further verify whether Gd-DCCP16 is covalently linked by transglutaminase activity, large MCF7 (human breast cancer cell line) spheroids (1 mm in diameter) were generated and incubated for 48 h with Gd-DCCP16 (1). Contrast enhancement was observed in T1-weighted images of spheroids incubated with Gd-DCCP16 in comparison to control spheroids.

Animal Studies

Rodents

[PubMed]

In vivo contrast enhancement with Gd-DCCP16 was investigated in nude mice bearing 4T1 (mouse mammary tumor cell line) tumors (n = 4 mice) (1). Two animals were intravenously injected with Gd-DCCP16 control, and the other two animals were injected with Gd-DCCP16. Images were acquired at 9.4 T before, immediately after, and 24 h after injection. The relaxation rate (R_1) was elevated in all tumors immediately after injection, but high R_1 was observed only for Gd-DCCP16 at 24 h after injection.

Other Non-Primate Mammals

[PubMed]

No references are currently available.

Non-Human Primates

[PubMed]

No references are currently available.

Human Studies

[PubMed]

No references are currently available.

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