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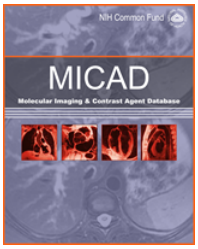

Gadolinium 1-((11-*S*)-3,10-diaza-13-carboxamido-11-carboxy-2,9-dioxotridecyl)-*N,N′,N″*-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane

Gd-DOTAMA-Gln

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Chemical name:	Gadolinium 1-((11-*S*)-3,10-diaza-13-carboxamido-11-carboxy-2,9-dioxotridecyl)-*N,N′,N″*-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane	
Abbreviated name:	Gd-DOTAMA-C6-Gln	
Synonym:		
Agent category:	Small molecule	
Target:	Glutamine transporter	
Target category:	Transporter	
Method of detection:	Magnetic Resonance Imaging (MRI)	
Source of signal/contrast:	Gadolinium	
Activation:	No	
Studies:	• *In vitro* • Rodents	No structure is available in PubChem.

Background

[PubMed]

Malignant cells that precede rapid cellular proliferation require an increasing supply of precursor amino acids to support protein and nucleotide biosynthesis (1). These substrates are translocated from the blood stream into cytoplasma (internalization) *via* highly efficient transporters that belong to a group of carrier proteins residing in the plasma membrane. After entering the cytoplasma, glutamine (Gln) is further transported into the mitochondria and hydrolyzed by phosphate-dependent glutaminase (2). As a main source of nitrogen, Gln circulates in the blood stream at 0.6–0.9 mmol/L and accumulates in some tissues in concentrations as high as 20 mmol/L (2). Tumors compete with surrounding tissues for nitrogen compounds, producing a negative

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nitrogen balance in host tissues and substantial nitrogen increase in tumors (3). Therefore, the evaluation of protein metabolism can be carried out *via* imaging of the Gln uptake in tumors (4, 5). Because the internalization of Gln is mediated by Na^+-dependent amino acid transporters, attaching a small gadolinium (Gd) chelate to Gln should not interfere with this receptor-mediated endocytosis process (RME) (5, 6). Although receptors normally are found in concentrations of 10^{-9} to 10^{-13} mol/g of tissue (1 μM to 10^{-4} μM), the RME mechanism recycles the receptor rapidly and results in a substantial ligand accumulation inside a cell in a short time (7). As an example, the ligands can be enriched to 10^{-8} to 10^{-9} mol/g in 1 h (7). For Gd chelates, this concentration meets the minimum requirement for magnetic resonance imaging (MRI) detection (8). The Gln transportation rate for tumors like hepatoma is 10–20 times faster than that for the healthy hepatocytes (1). Thus, the difference in Gln uptake can be used to distinguish between tumors and tissues *via* contrast agent–enhanced MRI (5).

Gadolinium 1-((11-*S*)-3,10-diaza-13-carboxamido-11-carboxy-2,9-dioxotridecyl)-*N,N',N"*-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane (Gd-DOTAMA-C6-Gln) is a functionalized Gln in which the amino group is linked to a Gd-1,4,7,10-tetraazacyclododecane-*N,N',N",N"'*- tetraacetic monoamide (Gd-DOTA) through a linear aliphatic chain of six carbons (-C_6H_{12}-) (5). Gd-DOTA moiety has a high thermodynamic stability that protects against the *in vivo* release of Gd ions (9). The aliphatic chain serves as a spacer between the DOTA coordination cage and the Gln moiety to reduce the hindrance of the whole complex and improve the interaction between the Gln moiety and the receptor on the cell membrane (5). The conjugation of Gd chelate to the amino group sacrifices one of the four Gln recognition points on its transporting proteins and prevents the transporting protein from proceeding with the successive steps that bring the Gln into the mitochondria (5). Thus, the transporter may move the Gd-DOTAMA-C6-Gln to the clathrin-rich region, where the chelates are trapped in the endosomal vesicles. The entrapment can cause a quenching effect in the relaxation enhancement because the endosomal membrane forms barriers for water molecules to interact with the paramagnetic centers (5). The small size of DOTAMA-C6-Gln allows for easy extravasation and diffusion into the tissue to reach the surface of tumor cells.

Synthesis

[PubMed]

Crich et al. reported a detailed synthesis of Gd-DOTAMA-C6-Gln (5). DOTAMA-C6-Gln was obtained by a coupling reaction between the N-hydroxysuccinimide (NHS) activated ester of 1-(3-aza-8-carboxy-2-oxooctyl)-*N,N',N"*-tris((1,1-dimethylethoxy)carbonylmethyl)-1,4,7,10-tetraazacyclododecane (DOTAMA(*t*BuO)$_3$-$(CH_2)_6$-OH) and the unprotected L-glutamine in a homogeneous mixture of acetonitrile and a phosphate buffer (pH 8). After deprotection of the *tert*-butyl esters in the presence of trifluoroacetic acid and CH_2Cl_2 (1:1) at 50% yield, the purified ligand formed a complex with $GdCl_3$ (pH 6.5).

The precursor 1-(3-aza-8-carboxy-2-oxooctyl)-4,7,10-tris(1,1-dimethylethoxy)-1,4,7,10-tetraazacyclododecane NHS (DOTAMA(*t*BuO)$_3$-$(CH_2)_6$-NHS) was prepared in multiple steps. First, 6-aminohexanoic acid benzyl ester was reacted with bromoacetyl bromide in the presence of K_2CO_3 in acetonitrile to yield 7-aza-9-bromo-8-oxononanoic acid benzyl ester at 90% yield. This product was converted to 1-(3-aza-8-carboxy-2-oxooctyl)-*N,N',N"*-tris((1,1-dimethylethoxy)carbonylmethyl)-1,4,7,10-tetraazacyclododecane benzyl ester at 96.5% yield by reacting it with 1,4,7,10-tetraazacyclododecane-*N,N',N"*-tris-*tert*-butylester (DO3A-tris-*tert*-butylester) in the presence of K_2CO_3 in acetonitrile. Then, the ester was hydrogenated in methanol under catalyzation of 10% Pd/C to produce DOTAMA(*t*BuO)$_3$-$(CH_2)_6$-OH in 50% yield, which was further converted to DOTAMA-$(CH_2)_6$-NHS by reacting with NHS in CH_2Cl_2.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The relaxation properties of water were examined with ^{1}H nuclear magnetic resonance (NMR) dispersion spectroscopy (NMRD) at different magnetic field strengths and with ^{17}O NMR spectroscopy at different temperatures (5). The structural water residual time (τ_m) was found to be 1.5 μs at 25°C, and the electronic relaxation time (τ_v) and the rotation correlation time (τ_R) were 25 and 83 ps, respectively. The T_1 relaxivity was found to be 5.5 $mM^{-1}s^{-1}$ at 0.47 T and 25°C in aqueous solution. For hepatoma cellular pellets with internalized Gd-DOTAMA-C6-Gln, the T_1 relaxivity was measured as a function of Gd per cell, which exhibited a substantial deviation from the theoretical calculation. This indicated that the internalization of the chelates occurred through the entrapment into endosomal vesicles.

The uptake of Gd-DOTAMA-C6-Gln in cells was tested in healthy rat hepatocytes, rat hepatoma cells (HTC), rat glioma C6 cells, murine breast adenocarcinoma cells (TSA), and murine neuroblastoma cells (Neuro-2a) (5). Two to three million cells were incubated for 6 h at 37°C in Earl's balanced salt solution (EBSS) containing Gd-DOTAMA-C6-Gln at 1.6 mM (three times higher than the glutamine physiological concentration). After incubation, the cells were washed three times and then destroyed. The Gd was released from the cells as free aquo-ions and quantified by inductively coupled plasma mass spectrometry (ICP-MS) to determine internalized Gd content (10). For all tumor cells, the internalized Gd was found to be $>1 \times 10^{-8}$ mol Gd /mg cell protein. The uptake of Gd-DOTAMA-C6-Gln by HTC cells appeared to decrease markedly as the concentration of the free Gln added to the culture medium increased. This result also demonstrated that Gln residue present on the surface of Gd-DOTAMA-C6-Gln complex was the vehicle for the internalization through the amino transporting system. The Gd-DOTAMA-C6-Gln uptake for HTC cells was further compared with that for hepatocytes by *in vitro* MRI on a 7-T imager. After 4 h of incubation in 1.6 mM of Gd-DOTAMA-C6-Gln, 23 nmol of Gd/mg cell protein was found in HTC cells, which was four times higher than the 5.5 nmol of Gd/mg cell protein in the hepatocytes. This difference was also reflected in the T_1-weighted images on which a 45% increase in signal intensity was found for the HTC cells compared to the healthy hepatocytes.

Animal Studies

Rodents

[PubMed]

MRI experiments were conducted to distinguish between tumors and normal tissues in two rodent tumor models (5). A dose of Gd-DOTAMA-C6-Gln (0.2 mmol/kg) was injected intravenously into A/J mice grafted with Neuro-2a and *her-2/neu* transgenic mice with mammary carcinoma. From 24 to 72 hr after injection, T_1-weighted images were acquired on a 7-T imager when the renal elimination of the non-internalized Gd complex was complete. The tumor signal intensity (SI) was significantly high, indicating that the amount of internalized Gd complex was sufficient to yield a substantial enhancement effect. The SI enhancement was homogeneously distributed in the tumor mass and was more pronounced in tumors with a diameter <3 mm. In comparison, the kidney cortical region showed ~15% SI enhancement. For the transgenic mouse model, the Gd uptake in the *her-2/neu* tumors was determined by ICP-MS. Tumors were extracted by euthanizing the mice 24 h after injection. The amount of Gd-DOTAMA-C6-Gln in tumors was found to be 2.1 ± 0.4 nmol Gd/g protein in tumors <3 mm in diameter and 1.2 ± 0.4 nmol Gd/g protein in tumors >3 mm in diameter. Compared to the muscles, the uptake of Gd-DOTAMA-C6-Gln was seven- to eight-fold higher for tumors <3 mm in diameter and four-fold higher for tumors >3 mm in diameter.

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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