Gd-DOTA-G-NH(CH_2)$_{11}$CO-RSPAYYTAA-(CH_2CH_2O)$_8$-R

GdPCA2

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Chemical name:	Gd-DOTA-G-NH(CH_2)$_{11}$CO-RSPAYYTAA-(CH_2CH_2O)$_8$-R	
Abbreviated name:	GdPCA2	
Synonym:	PCA2-switch	
Agent category:	Peptide	
Target:	Matrix metalloprotein-2 (MMP-2)	
Target category:	Enzyme	
Method of detection:	Magnetic Resonance Imaging (MRI)	
Source of signal/contrast:	Gadolinium	
Activation:	Yes	
Studies:	• *In vitro* • Rodents	No structure is current available in PubChem.

Background

[PubMed]

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases localized at the cell surface or in extracellular compartments (1). MMPs degrade all components of the extracellular matrix (ECM) and are associated with a variety of pathological conditions such as wound healing, tissue remodeling, tumor angiogenesis, and embryo development. The active site of MMPs contains a catalytic domain coordinated by zinc to recognize motifs with a consensus sequence of PXX↓X_{Hy}, where ↓ represents the cleavage point and X_{Hy} represents a large hydrophobic residue (2). Excess

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MMP activity has been observed in conjunction with many diseases, including rheumatoid arthritis, osteoarthritis, autoimmune diseases, cardiovascular diseases, and cancer (3). For example, an overexpression of MMP subtype-2 (MMP-2 or gelatinase A), an enzyme that degrades type IV collagen and gelatin, is present in many human tumors (4). Thus, MMP has been an important therapeutic target for many years (5).

Gadolinium (Gd)-labeled DOTA-G-NH$(CH_2)_{11}$CO-RSPAYYTAA-$(CH_2CH_2O)_8$-R (GdPCA2) is a proteinase-modulated contrast agent (PCA) for *in vivo* imaging of MMP-2 with magnetic resonance imaging (MRI) (6). GdPCA2 consists of four components: a peptide substrate (RSPAY↓YTAA) specific for MMP-2, a Gd-DOTA complex as an MRI probe, an alkyl chain of 12-carbon as a hydrophilic linker between the N-terminus of the peptide and the MRI probe, and an eight-unit polyethylene glycol (PEG_8) chain linked to the C-terminus of the peptide to enhance the solubility of GdPCA2. The cleavage of GdPCA2 by MMP-2 produces a less soluble Gd^{3+}-labeled fragment. Thus, the Gd species acts as a solubility switch specific for the enzyme MMP-2. GdPCA2 may have different pharmacokinetics than its cleaved product, which can be used to evaluate MMP-2 activity *via* dynamic MRI measurement.

Synthesis

[PubMed]

Lebel et al. briefly described the preparation of GdPCA2 (6). With standard protocols of solid-phase peptide synthesis, the N-terminus of the PCA2 peptide (RSPAYYTAA) was linked to the Gd-DOTA *via* a 12-carbon alkyl chain, and the C-terminus of the peptide was linked to an amino PEG_8-Arg to yield GdPCA2.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The cleavage efficacy (k_{cat}/K_m) of GdPCA2 was measured *in vitro* (6). Cleavage of GdPCA2 by MMP-2 produced a free amino group that was detectable with the addition of fluorescamine. The k_{cat}/K_m was 1.2×10^5 $M^{-1}s^{-1}$ for GdPCA2, which was slightly lower than the k_{cat}/K_m of 3.1×10^5 $M^{-1}s^{-1}$ for the PCA2 peptide but 37.5 times higher than the k_{cat}/K_m of 3.1×10^5 $M^{-1}s^{-1}$ for a scrambled GdPCA2 (GdPCA2-scrambled) that contained a scrambled peptide (SYPATAYA). The cleaved products were further examined with high-performance liquid chromatography and mass spectrometry; two fragments were found for GdPCA2 (specific cleavage) and four fragments for GdPCA2-scrambled (non-specific cleavage).

The T_1 relaxivity of GdPCA2 and its cleaved product (cleaved-GdPCA2) was measured at 7 T (6). The value for the GdPCA2 was 2.06 ± 0.03 $mM^{-1}s^{-1}$ in H_2O and 2.03 ± 0.03 $mM^{-1}s^{-1}$ in aqueous bovine albumin (BSA) (14 mg/ml), respectively. The values for the cleaved-GdPCA2 were slightly higher: 2.18 ± 0.03 $mM^{-1}s^{-1}$ in H_2O and 2.19 ± 0.02

$mM^{-1}s^{-1}$ in aqueous BSA. In comparison, the T_1 relaxivity of GdDTPA was 3.58 ± 0.10 $mM^{-1}s^{-1}$.

Animal Studies

Rodents

[PubMed]

Lebel et al. used GdPCA2 with dynamic contrast-enhanced MRI to examine the MMP-2 activity as a function of time (the pharmacokinetics) *in vivo* (6). Balb/c mice were implanted with two types of mammary carcinomas ~310 mm^3 in volume: a wild-type MC7-L1 (WT) tumor that had overexpressed MMP-2 at the left hind limb and a knockdown MC7-L1 (KD) tumor that had suppressed MMP-2 (~51% lower) at the right hind limb. The tumor-bearing mice (n = 8) were injected with 2 μmol GdPCA2 *via* the caudal vein, and sequential T_1-weighted images were collected at 7 T at a temporal resolution of 51 s. The signal in each voxel was converted into the relaxation rate difference (ΔR_1) between the relaxation rate R_1 and the precontrast relaxation rate $R_{1,0}$. A rapid increase in ΔR_1 was observed in the WT and KD tumors after the injection of GdPCA2, but the subsequent pharmacokinetics were different in the two types of tumors. In the WT tumor, ΔR_1 remained constant 5–20 min after injection, then exhibited a second increase with a maximum at ~40 min. In the KD tumor, ΔR_1 continued to decrease 5 min after injection. As a control, the tumor-bearing mice (n = 2) were injected with 2 μmol GdPCA2-scrambled and imaged with the same MRI protocols. A pharmacokinetics similar to that of the KD tumor after injection of GdPCA2 was observed in both the WT tumor and the KD tumor after injection of GdPCA2-scrambled. This result suggests that the GdPCA2 in WT tumors is different from the GdPCA2-scrambled in both tumors. In the WT tumor, the first increase in ΔR_1 after injection of GdPCA2 was caused by the perfusion of GdPCA2 from the blood to the ECM, as seen in all tumors and/or injection with GdPCA2-scrambled; the second increase was attributed to the activation of enzyme MMP-2, which was only present in tumors overexpressing MMP-2 (WT type).

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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