Gadolinium-diethylenetriamine pentaacetic acid-GKWHCTTKFPHHYCLY

EP-3533

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Chemical name:	Gadolinium-diethylenetriamine pentaacetic acid-GKWHCTTKFPHHYCLY	
Abbreviated name:	EP-3533	
Synonym:		
Agent category:	Peptide	
Target:	Collagen type I	
Target category:	Acceptor	
Method of detection:	Magnetic resonance imaging (MRI)	
Source of signal/contrast:	Gadolinium, Gd	
Activation:	No	
Studies:	• *In vitro* • Rodents	No structure is available in PubChem.

Background

[PubMed]

Magnetic resonance imaging (MRI) maps information about tissues spatially and functionally. Protons (hydrogen nuclei) are widely used in imaging because of their abundance in water molecules. Water comprises ~80% of most soft tissue. The contrast of proton MRI depends primarily on the density of the nucleus (proton spins), the relaxation times of the nuclear magnetization (T1, longitudinal; T2, transverse), the magnetic environment of the tissues, and the blood flow to the tissues. However, insufficient

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contrast between normal and diseased tissues requires the development of contrast agents. Most contrast agents affect the T1 and T2 relaxation times of the surrounding nuclei, mainly the protons of water. T2* is the spin–spin relaxation time composed of variations from molecular interactions and intrinsic magnetic heterogeneities of tissues in the magnetic field (1). Cross-linked iron oxide (CLIO) nanoparticles and other iron oxide formulations affect T2 primarily and lead to decreased signals. On the other hand, paramagnetic T1 agents such as gadolinium (Gd^{3+}) and manganese (Mn^{2+}) accelerate T1 relaxation and lead to increased contrast images.

Extracellular matrix adhesion molecules consist of a complex network of fibronectins, collagens, chondroitins, laminins, glycoproteins, heparin sulfate, tenascins, and proteoglycans that surround connective tissue cells, and they are mainly secreted by fibroblasts, chondroblasts, and osteoblasts (2). Cell substrate adhesion molecules are considered essential regulators of cell migration, differentiation, and tissue integrity and remodeling. These molecules play a role in inflammation and atherogenesis, but they also participate in the process of invasion and metastasis of malignant cells in the host tissue (3). Fibrosis is the formation of excess fibrous connective tissue (mainly collagen type I) in an organ or tissue as a reparative or reactive process in many chronic diseases in the heart, liver, kidneys, lungs, or vasculatures.

Gd, a lanthanide metal with seven unpaired electrons, has been shown to be very effective in enhancing proton relaxation because of its high magnetic moment and water coordination (4, 5). Gd-Labeled diethylenetriaminepentaacetic acid (Gd-DTPA) was the first intravenous MRI contrast agent used clinically, and a number of similar Gd chelates have been developed in an effort to further improve clinical use. However, these low molecular weight Gd chelates have short blood and tissue retention times, which limit their use as imaging agents in the vasculature and in cancer tissues. Furthermore, they are largely nonspecific. GKWHCTTKFPHHYCLY, a collagen type I binding peptide, was identified with phage display screening (6). Subsequent addition of biphenylalanine at the amidated C terminus improved collagen binding. The peptide was conjugated with three moieties of Gd-DTPA (Gd-DTPA) to form Gd-DTPA-GKWHCTTKFPHHYCLY (EP-3533) for imaging of collagen in fibrotic lesions (6, 7).

Synthesis

[PubMed]

$GdCl_3$•6 H_2O (2.65 mmol) was added to a solution of 2-(4-isothiocyanatobenzyl)-DTPA (ITC-DTPA, 2.65 mmol; pH 6) to form ITC-Gd-DTPA (6). GKWHCTTKFPHHYCLY (0.0133 mmol, borate buffer (pH 9)), prepared with solid-phase synthesis, was incubated with 0.18 mmol ITC-Gd-DTPA for 69 h at room temperature. The product, EP-3533, was isolated with high-performance liquid chromatography with a yield of 27%. There were three Gd molecules per EP-3533 molecule. The mass of EP-3533 was confirmed with mass spectroscopy. EP-3612 was also prepared similarly with ITC-Gd-DTPA using an inactive peptide (GKWHCTTKFPHHYCLY).

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

EP-3533 exhibited r_1 relaxivity values of 83.7 $mM^{-1}s^{-1}$ at 0.47 T and 46.8 $mM^{-1}s^{-1}$ at 1.41 T at 37°C in human plasma (6). EP-3612 showed values similar to EP-3533. The r_2 relaxivity values were 97.5 and 101.7 $mM^{-1}s^{-1}$ at 1.41 T for EP-3533 and EP-3612, respectively. On the other hand, the relaxivity of these agents is ~15-fold higher than that of Gd-DTPA. EP-3533 exhibited a K_d value of 1.8 μM for collagen type I with eight binding sites per collagen molecule, whereas EP-3612 showed a K_d value of 400 μM.

Animal Studies

Rodents

[PubMed]

Caravan et al. (6) performed biodistribution studies in normal mice (n = 4). The organ with the highest uptake was the kidney (223 nmol Gd/g), followed by the spleen (77 nmol Gd/g), liver (50 nmol Gd/g), lung (29 nmol Gd/g), heart (26 nmol Gd/g), blood (14 nmol Gd/g), and femur (2 nmol Gd/g) at 15 min after injection of EP-3533 (10 μmol/kg). On the other hand, EP-3612 exhibited Gd levels in the blood and femur similar to EP-3533 but markedly lower levels in the other organs. MRI images (1.4 T) of collagen in a mouse model of healed myocardial infarction showed that EP-3533 enhanced T1-weighted images in the collagen-rich scar at 40 min after injection, whereas no such enhancement was observed with EP-3612. No blocking experiment was performed.

Helm et al. (7) performed dynamic T1-weighted MRI (4.7 T) studies with EP-3533 in mice (n = 8) with healed myocardial infarction. The washout time constants for EP-3533 were significantly longer than those for Gd-DTPA in regions of postinfarction scarring (194.8 ± 116.8 min *versus* 25.5 ± 4.2 min; $P < 0.05$) and in normal myocardium (45.4 ± 16.7 min *versus* 25.1 ± 9.7 min; $P < 0.05$). The washout time constants for EP-3533 and Gd-DTPA were similar in the blood (15.0 ± 2.7 min *versus* 17.9 ± 4.2 min, respectively). Histological staining for collagen correlated well with EP-3533–enhanced areas on MRI images. The scar tissue samples had a two-fold higher concentration of Gd than the normal myocardium at 50 min after injection. No blocking experiment was 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.

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