Folate-polyethylene glycol-ultrasmall superparamagnetic iron oxide nanoparticles

P1133

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Chemical name:	Folate-polyethylene glycol-ultrasmall superparamagnetic iron oxide nanoparticles	
Abbreviated name:	P1133	
Synonym:		
Agent category:	Compound	
Target:	Folate receptor	
Target category:	Receptor	
Method of detection:	Magnetic resonance imaging (MRI)	
Source of signal:	Iron oxide	
Activation:	No	
Studies:	• *In vitro* • Rodents	Structure is not 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, which comprise ~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; and 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 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 brighter contrast images.

The superparamagnetic iron oxide (SPIO) structure is composed of ferric iron (Fe^{3+}) and ferrous iron (Fe^{2+}). The iron oxide particles are coated with a protective layer of dextran or another polysaccharide. These particles have large combined magnetic moments or spins, which are randomly rotated in the absence of an applied magnetic field. SPIO is used mainly as a T2 contrast agent in MRI, though it can shorten both T1 and T2/T2* relaxation processes. SPIO particle uptake into the reticuloendothelial system (RES) is by endocytosis or phagocytosis. SPIO particles are also taken up by phagocytic cells such as monocytes, macrophages, and oligodendroglial cells. A variety of cells can also be labeled with these particles for cell trafficking and tumor-specific imaging studies. SPIO agents are classified by their sizes with coating material, which can range from ~10 to 3,500 nm in diameter, as large SPIO nanoparticles, standard SPIO nanoparticles, ultrasmall SPIO (USPIO) nanoparticles, and monocrystalline iron oxide nanoparticles (1).

Folic acid is a water-soluble B vitamin (2) that is essential for methylation and DNA synthesis. The primary pathway for folate entry into cells is through the facilitated transporter, which has a low affinity for folate (Michaelis constant (K_m) = 1–5 μM). Some cells in the choroid plexus, kidney, lung, thyroid, spleen, placenta, and thymus also possess a higher-affinity receptor (dissociation constant (K_d) = 0.5 nM) that allows folate uptake *via* receptor-mediated endocytosis. Some human epithelial tumor cells have been found to overexpress folate-binding protein (3). More than 90% of human ovarian and endometrial cancers express the high-affinity receptor, which is absent in the respective normal tissues. Breast, colorectal, renal, and lung carcinomas also overexpress the folate receptor but at lower frequencies (20%–50%). Activated macrophages, but not resting macrophages, have also been found to express the folate receptor (4). Several folate-based conjugates have been studied in tumor imaging [PubMed].

USPIO iron nanoparticles have diameters of 4–6 nm, and the hydrodynamic diameters with dextran or polyethylene glycol (PEG) coating are 20–50 nm. USPIO nanoparticles have a long plasma half-life because of their small size. The blood pool half-life is calculated to be ~24 h in humans (5) and 2 h in mice (6). Because of its long blood half-life, USPIO can be used as a blood pool agent during the early phase of intravenous administration (7). In the late phase, USPIO is suitable for the evaluation of the RES in the body, particularly in the lymph nodes (8). Various ligands and antibody-conjugated USPIO nanoparticles have been studied for *in vivo* targeted MRI in small animals (9, 10). Meier et al. (11) performed *in vivo* MRI of breast cancer in mice using folate-conjugated PEG-USPIO nanoparticles (P1133).

Related Resource Links:

- Chapters in MICAD (Folate)
- Gene information in NCBI (Folate receptor)
- Articles in Online Mendelian Inheritance in Man (OMIM) (Folate receptor)
- Clinical trials (Folate receptor, USPIO)
- Drug information in FDA (Folate receptor)

Synthesis

[PubMed]

Meier et al. (11) prepared folate-PEG-amine by reaction of folic acid with PEG-bis-amine in anhydrous dimethyl sulfoxide with diisopropylcarbodiimide/*N*-hydroxysuccinimide. Folate-PEG-amine was purified with column chromatography. Folate-PEG-amine was added to carboxylate-USPIO nanoparticles. The product, P1133, had a mean hydrodynamic diameter of 26 nm. There were 8–10 folate moieties per nanoparticle. P904 (PEG-USPIO without folate; diameter, 21 nm) was also prepared as a control. P1133 exhibited an r1 relaxivity value of 12 $mM^{-1}s^{-1}$ and an r2 relaxivity value of 95 $mM^{-1}s^{-1}$ in water at 60 MHz. P904 showed r1 and r2 relaxivity values similar to those of P1133.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Cells from human breast cancer cell line MDA-MB-231 express high levels of mRNA for folate receptor, whereas cells from the human lung carcinoma A549 have no detectable level (11). MDA-MB-231 cells (10×10^6) were incubated with 50 μM P1133 and P904 with or without 750 μM folate for 24 h. P1133-Labeled MDA-MB-231 cells showed an iron uptake of 8.2 pg/cell, which was inhibited to 4.7 pg/cell with excess folate ($P = 0.002$). P904-Labeled cells (with or without excess folate) showed an iron uptake of 1.5 pg/cell. Untreated control cells showed iron levels of 0.68–0.73 pg/cell. The cell viability of P1133-labeled cells (95 ± 3%) was not significantly different from that of P904-labeled cells (97 ± 2%). MRI showed that P1133-labeled cells exhibited R2 relaxation rates of 0.0172 ms^{-1} and 0.0116 ms^{-1} without and with excess folate, respectively. Excess folate blocked uptake of P1133. P904-Labeled cells (with or without excess folate) showed R2 relaxation rates of ~0.0075–0.0079 ms^{-1}, indicating that folate had little effect on the control P904. Untreated control cells showed R2 relaxation rates of 0.0071–0.0075 ms^{-1}.

Animal Studies

Rodents

[PubMed]

Meier et al. (11) performed a series of whole-body 3-T MRI (T2-weighted) in nude mice ($n = 6$) bearing MDA-MB-231 or A549 tumors after intravenous injection of 0.5 mmol Fe/kg P1133 and P904. Folate receptor–positive MDA-MB-231 tumors showed a moderate decline ($P < 0.05$) in signal/noise ratio (SNR) during the early phase (1 h) after injection of P1133 (21.1 ± 6.2) and P904 (19.7 ± 3.2) as compared with pre-injection (P1133 = 28.9 ± 6.0 and P904 = 27.4 ± 3.9). At 24 h, there was a persistent negative enhancement with P1133 (SNR = 14.9 ± 2.5, $P = 0.011$) but not with P904 (SNR = 27.0 ± 3.8, $P = 0.29$). The early-phase (1 h) negative enhancement was due to non-target perfusion retention in the tumors, and the later-phase (24 h) enhancement was due to P1133 targeting the folate receptor–positive tumors. On the other hand, folate receptor–negative A549 tumors showed a minor negative enhancement during the early phase and did not show a persistent negative enhancement during the later phase. Immunohistochemical staining confirmed the presence of folate receptors in MDA-MB-231 tumors and the absence of folate receptors in A549 tumors. P1133-Treated MDA-MB-231 tumors exhibited intracellular and extracellular retention of iron particles, but the same was not observed in P1133-treated A549 tumors, P904-treated MDA-MD-231 tumors, or P904-treated A549 tumors.

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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