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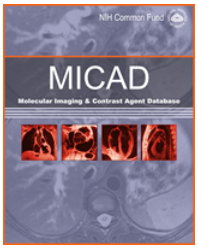

Malachite green-isothiocyanate-polyethylene glycol-gold nanoparticles conjugated with scFv anti-EGFR B10 antibody

MGITC-AuNPs-scFvB10

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Chemical name:	Malachite green isothiocyanate-polyethylene glycol-gold nanoparticles conjugated with scFv anti-EGFR B10 antibody	
Abbreviated name:	MGITC-AuNPs-scFvB10	
Synonym:		
Agent Category:	Metal	
Target:	EGFR, HER1	
Target Category:	Antibody-antigen binding	
Method of detection:	Optical, surface-enhanced Raman scattering	
Source of signal:	Malachite green	
Activation:	No	
Studies:	• *In vitro* • Rodents	No structure is currently available in PubChem.

Background

[PubMed]

Optical fluorescence imaging is increasingly used to monitor biological functions of specific targets in small animals (1, 2). However, the intrinsic fluorescence of biomolecules poses a problem when fluorophores that absorb visible light (350–700 nm) are used. Near-infrared (NIR) fluorescence (700–1,000 nm) detection avoids the background fluorescence interference of natural biomolecules, providing a high contrast between target and background tissues. NIR fluorophores have a wider dynamic range and minimal background as a result of reduced scattering compared with visible fluorescence detection. They also have high sensitivity, resulting from low infrared background, and high extinction coefficients, which provide high quantum yields. The NIR region is also compatible with solid-state optical components, such as diode lasers and silicon detectors. NIR fluorescence (NIRF) imaging is becoming a non-invasive alternative to radionuclide imaging in small animals.

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Hainfeld et al. (3) performed experiments in mice using gold nanoparticles (AuNPs; 1.9 nm in diameter, ~50 kDa) as a computed tomography contrast agent to enhance imaging contrast in the vasculature, kidneys, and tumor tissue in mice. No physiological complications were observed in the mice for up to a year. James et al. (4) showed that gold nanoshells (110 nm in diameter) coated with polyethylene glycol (PEG) had no toxic effects in mice for up to 28 days. PEG is found to minimize nonspecific adsorption of proteins onto nanoparticles and to reduce their uptake by the liver (5). Surface-enhanced Raman scattering (SERS) involves surface plasmons of silver and gold as excited by a NIR laser (785 nm), and the resulting electric fields cause other nearby molecules (reporter molecules) to become Raman active (6, 7). The result is amplification of the Raman signals (by up to 10^{14}), allowing detection and identification of single molecules. Qian et al. (8) found that SERS PEG-AuNPs (60–80 nm) are >200 times brighter (NIR signal) than quantum dots on a particle-to-particle basis. A single-chain anti-EGFR B10 antibody fragment (~25 kDa) consisting of the variable V_H and V_L domains for antigen binding (scFvB10) was conjugated to SERS nanoparticles (MGITC-AuNPs-scFvB10) for tumor imaging in a small animal model. The reporter molecules are malachite green isothiocyanate (MGITC). MGITC-AuNPs-scFvB10 has electronic transitions at 633 nm or 785 nm.

Synthesis

[PubMed]

Qian et al. (8) reported the preparation MGITC-AuNPs-scFvB10. A solution of 3–4 μM of MGITC was added slowly to a rapidly stirred gold colloid solution. After 10 min, a solution of thio-PEG (10 μM) was added dropwise to the MGITC-AuNP solution. The MGITC-AuNP solution was washed and isolated by centrifugation. The core size of each MGITC-AuNP was ~60 nm with >30,000 copies of thio-PEG (5 kDa). For protein conjugation, a solution of thio-PEG-COOH (1 μM) was added slowly to another freshly prepared MGITC-AuNP solution. After 15 min, a large excess of thio-PEG was added to coat the area not covered by the thio-PEG-COOH. A solution of ethyl dimethylaminopropyl carbodiimide and sulfo-*N*-hydroxysuccinimide was added to activate the carboxyl groups of PEG-COOH on the MGITC-AuNPs. The activated nanoparticles were incubated with scFv B10 antibody (11.2 nmol) for 20 h. MGITC-AuNPs-scFvB10 was isolated and purified by centrifugation and membrane filtration to remove the unreacted antibody. There were ~600 copies of scFv B10 antibody/nanoparticle; there were $1.4–1.5 \times 10^4$ MGITC molecules/nanoparticle. MGITC-AuNPs-scFvB10 had a hydrodynamic diameter of ~80 nm. MGITC-AuNPs-scFvB10 has electronic transitions at 633 nm for NIRF imaging and 785 nm for NIR SERS imaging; therefore, MGITC-AuNPs-scFvB10 is a multimodal agent. Another reporter dye (diethylthiatricarbocyanine (DTTC)) conjugated with AuNPs-scFvB10 (DTTC-AuNPs-scFvB10) was also prepared similarly.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Qian et al. (8) performed cell-binding assays with DTTC-AuNPs-scFvB10 using human head-and-neck carcinoma Tu866 (HER1-positive) and non-small cell lung carcinoma NCI-H520 (HER1-negative) cell lines. SERS measurements at 30 min of incubation with DTTC-AuNPs-scFvB10 (15 pM) showed that Tu866 cells exhibited strong SERS signals, whereas H520 cells showed little or no signal. Pretreatment of Tu866 cells with a ten-fold excess of scFvB10 abrogated the binding of DTTC-AuNPs-scFvB10 to the cells. No signal was obtained after incubation of Tu866 cells with DTTC-AuNPs-IgG (nonspecific antibody) or DTTC-AuNPs (control).

Animal Studies

Rodents

[PubMed]

Qian et al. (8) performed studies in which MGITC-AuNPs (0.05 pmol) were injected subcutaneously in a nude mouse (1–2 mm under the skin and 1 cm deep in muscle). NIR Raman spectroscopy (full width at half maximum = 1–2 nm) was used to measure SERS spectra at the two locations at 3 and 21 s after injection. The highly resolved SERS signals obtained were identical to the reference MGITC-AuNPs spectrum, although the intensities were one- to two-fold lower. It was estimated that SERS signals could be detected as deep as 2 cm into tissue. In another experiment, either MGITC-AuNPs-scFvB10 or MGITC-AuNP (460 pmol) was injected intravenously into nude mice (n = 4) bearing a Tu866 xenograft tumor (3 mm in diameter). NIR Raman spectroscopy of the tumor and liver *in vivo* was performed at 5 h. There were substantial differences in the tumor signal intensities between the targeted and non-targeted nanoparticles. In contrast, the SERS signal intensities were similar in the liver. The tumor SERS signal intensities of targeted nanoparticles were ~three-fold greater than those of the liver and stayed in the tumor for >24 h. The biodistribution of the nanoparticles was measured by inductively coupled plasma-mass spectroscopy at 5 h after injection. The excised tissue with the highest MGITC-AuNPs-scFvB10 accumulation was the spleen (40 ppm), followed by the liver (14 ppm) and the tumor (9 ppm). There was minimal accumulation in the kidney, muscle, and lung. MGITC-AuNPs and MGITC-AuNPs-IgG had similar biodistribution values as compared with MGITC-AuNPs-scFvB10, with the exception of the tumor (<1 ppm for MGITC-AuNPs and MGITC-AuNPs-IgG). AuNPs were taken up by the reticuloendothelial system and phagocytes in the liver and spleen. Transmission electron microscopy images of the tumor cells showed intracellular clusters of MGITC-AuNPs-scFvB10 in intracellular endosomes and lysosomes. On the other hand, the liver Kupffer cells and spleen macrophages showed intracellular accumulation of single nanoparticles in these organelles. No signs of sickness were observed in the mice injected with these nanoparticles for up to 2–3 months. No blocking experiments were 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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