^{99m}Tc-Labeled acetylated dendrimer poly(amido)-amine generation 5-folic acid-2-(*p*-isothiocyanatobenzyl)-6-methyl-diethylenetriamine pentaacetic acid conjugate

^{99m}Tc-G5-Ac-FA-DTPA

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Chemical name:	^{99m}Tc-Labeled acetylated dendrimer poly(amido)-amine generation 5-folic acid-2-(*p*-isothiocyanatobenzyl)-6-methyl-diethylenetriamine pentaacetic acid conjugate	
Abbreviated name:	^{99m}Tc-G5-Ac-FA-DTPA	
Synonym:	^{99m}Tc-Ac-G5-FA-1B4M DTPA	
Agent Category:	Compounds	
Target:	Folate receptor	
Target Category:	Receptor	
Method of detection:	Single-photon emission computed tomography (SPECT); gamma planar imaging	
Source of signal / contrast:	^{99m}Tc	
Activation:	No	
Studies:	• *In vitro* • Rodents	No structure is available.

Background

[PubMed]

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^{99m}Tc-Labeled acetylated (Ac) dendrimer poly(amido)-amine (PAMAM) generation 5 (G5)-folic acid (FA)-2-(*p*-isothiocyanatobenzyl)-6-methyl-diethylenetriamine pentaacetic acid (1B4M DTPA) conjugate, abbreviated as ^{99m}Tc-G5-Ac-FA-DTPA, was synthesized by Zhang et al. for folate receptor (FR)-targeted imaging of FR-positive tumors (1, 2).

Folate is an essential vitamin for cell synthesis of nucleotide bases. Some unique features of the FR-folate system make it extremely valuable for developing FR-targeted imaging and therapeutic agents (3-5). First, FR has a high affinity for the exogenous folate conjugates (K_d = ~100 pM), but they are inaccessible for the conjugates in most normal tissues. Second, FR-α isoform is overexpressed on ~40% of human cancers, where it is completely accessible to folate conjugates. Third, folate conjugates are taken up by cancer cells *via* FR-mediated endocytosis, and FR recycles actively to the cell surface with a frequency of 5.7–20 h depending on cell types. Fourth, conjugation of imaging labels *via* the γ-carboxyl group of folate has no apparent effects on the ligand-binding affinity to FR. Furthermore, folate is a small molecule (MW = ~441) that exhibits rapid and complete penetration of solid tumors and rapid clearance from FR-negative tissues ($t_{1/2}$, <10 min). One disadvantage of the FR-folate system is that FR expresses at a relatively low density on some tumor cell surface (1–3 million FR/cell), which means that the FR-folate binding can be saturated rapidly and the imaging contrast will be limited (3, 4, 6). Sensitive imaging techniques such as positron emission tomography and single-photon emission computed tomography (SPECT) are more desirable for FR-targeted imaging (3, 4, 7). To date, a large set of folate-based radiopharmaceutical agents have been synthesized for nuclear imaging (8). In general, a chelating agent is necessary to bridge the radiolabels and the folate molecule. The chelating agent is also critical for optimizing the molecular properties of conjugates (1, 2, 9).

Dendrimers represent a unique class of nanostructures that are synthesized from branched monomers in a step-wise manner. The molecular properties of dendrimers can be precisely controlled by choosing different branching monomers and surface functional groups (1, 2, 9). PAMAM is one of the most extensively studied dendrimers. Zhang et al. synthesized two folate-based compounds, ^{99m}Tc-G5-Ac-pegFA-DTPA and ^{99m}Tc-G5-Ac-FA-DTPA, and one control, ^{99m}Tc-G5-Ac-DTPA, with PAMAM G5 dendrimer and folate (1, 2). To increase the solubility and decrease the nonspecific cellular uptake of the agents, the primary amines on the surface of PAMAM dendrimers were partially converted to acetamide moieties, which were used to link with the bifunctional chelating agent 1B4M DTPA. Folate was either PEGylated or left unPEGylated, and its c-carboxyl group was used to conjugate with the primary amine of PAMAM. The two folate-based conjugates exhibited excellent stability, rapid clearance from blood, and high accumulation in tumor xenografts (1, 2). This chapter describes the results obtained with ^{99m}Tc-G5-Ac-FA-DTPA. Another chapter in MICAD describes the data obtained with ^{99m}Tc-G5-Ac-pegFA-DTPA.

Related Resource Links:

- FR-targeted chapters on MICAD

- Protein and nucleotide information of FR
- FR-targeted clinical trials in Clinicaltrials.gov

Synthesis

[PubMed]

Zhang et al. described the synthesis of ^{99m}Tc-G5-Ac-pegFA-DTPA, ^{99m}Tc-G5-Ac-FA-DTPA, and ^{99m}Tc-G5-Ac-DTPA (1, 2). The primary amines on the surface of G5 PAMAM dendrimers were first partially converted to acetamide moieties (G5-Ac, yield = 94.7%). PEGylated folate (NHS-PEG1540-FA) was synthesized through reactions of *N*-hydroxysuccinimide ester of FA with poly(ethylene glycol)bis amine (NH_2-PEG1540-NH_2). Conjugation of the folate or PEGylated folate to the dendrimers was achieved *via* condensation between the γ-carboxyl group of folate and the primary amine of dendrimer (G5-Ac-FA or G5-Ac-pegFA, yield = 90.8%). The partially acetylated dendrimers were then linked with the chelating agent 1B4M DTPA to generate G5-Ac-FA-DTPA, G5-Ac-pegFA-DTPA, or G5-Ac-DTPA (yield = 98.2%). Each G5-Ac-DTPA contained 77 acetyl and 8 DTPA molecules. Each G5-Ac-FA-DTPA contained 77 acetyl, 4.8 folic acid molecules, and 7 DTPA molecules, and each G5-Ac-pegFA-DTPA contained 77 acetyl, 5.2 folic acid molecules, and 8 DTPA molecules. Therefore, the three compounds (G5-Ac-pegFA-DTPA, G5-Ac-FA-DTPA, and G5-Ac-DTPA) contained a similar number of DTPA molecules (i.e., 7 or 8 molecules). The two folate conjugates (G5-Ac-pegFA-DTPA and G5-Ac-FA-DTPA) contained a similar number of folic acid molecules (i.e., 4.8 to 5.2 molecules).

Radiolabeling was performed after reduction of ^{99m}TcO4– to reduced ^{99m}Tc using stannous chloride. The radiochemical yield and radioactivity of the three radiolabeled agents were >95% and 88.3 ± 1.0%, respectively. They were used for experiments without further purification. The partition ratios (log *P*) between n-octanol and water were −2.030, −2.061, and −2.167 for ^{99m}Tc-G5-Ac-FA-DTPA, ^{99m}Tc-G5-Ac-pegFA-DTPA, and ^{99m}Tc-G5-Ac-DTPA, respectively, indicating that the agents had high water solubility.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The *in vitro* stability was analyzed after incubation of the ^{99m}Tc-G5-Ac-FA-DTPA, ^{99m}Tc-G5-Ac-pegFA-DTPA, and ^{99m}Tc-G5-Ac-DTPA with phosphate-buffered saline and new-born calf serum, respectively. After incubation for 6 h at 37°C, at least 86% and 84% of the agents kept their original structure in phosphate-buffered saline and in new-born calf serum, respectively, indicating high stability.

Cell uptake of the three agents was quantitatively measured with FR-expressing KB cells (a human oral epidermoid carcinoma line) (1, 2). *In vitro* cell binding of the ^{99m}Tc-G5-Ac-pegFA-DTPA was ~15% of total added radioactivity after incubation for 6 h at 37°C,

whereas ^{99m}Tc-G5-Ac-FA-DTPA and the control ^{99m}Tc-G5-Ac-DTPA exhibited lower binding ability (~11% and ~10%, respectively).

These results indicated that conjugation of FA directly to PAMAM dendrimers did not improve the cell uptake. In contrast, conjugation through a PEG spacer could improve the cellular internalization. Uptake of the three agents in the KB cells grown in FA-rich medium (to inhibit FR expression) was similar (<10%), suggesting no significant binding.

Animal Studies

Rodents

[PubMed]

The *in vivo* studies on stability after injection into mice (n = 3/time point) showed that 80.45% of the ^{99m}Tc-G5-Ac-FA-DTPA, and 80.56% of the ^{99m}Tc-G5-Ac-DTPA remained intact within 6 h in blood of normal mice (1, 2).

The pharmacokinetics of the agents was studied in normal mice (n = 5). A rapid decrease was observed at 30 min after injection, followed by a slow clearance. The plasma half-life was estimated to be 13.75 min for the ^{99m}Tc-G5-Ac-FA-DTPA and 12.73 min for the control ^{99m}Tc-G5-Ac-DTPA. Less than 10% of the injected agents remained in circulation after 6 h (assuming that blood represents 5.5% of the total body mass).

Biodistribution was investigated with KB tumor–bearing nude mice (n = 3/time point). The mice were maintained on a folate-deficient diet for the duration of experiment to minimize the circulating levels of FA. Both ^{99m}Tc-G5-Ac-DTPA and ^{99m}Tc-G5-Ac-FA-DTPA were cleared rapidly from the blood, decreasing from 11.75% injected dose/gram (ID/g) at 2 h to 5.60% ID/g at 6 h for ^{99m}Tc-G5-Ac-DTPA and from 12.59% ID/g at 2 h to 4.00% ID/g at 6 h for ^{99m}Tc-G5-Ac-FA-DTPA. Both agents remained at a low level up to 6 h in the FR-negative organs, including brain.

The kidneys, which express FR, are the major clearance organs. The level of nontargeted ^{99m}Tc-G5-Ac-DTPA decreased from 32.08% ID/g at 2 h to 26.06% ID/g at 6 h. In contrast, the level of ^{99m}Tc-G5-Ac-FA-DTPA increased slightly with time (22.06% ID/g at 2 h to 28.18% ID/g at 6 h). In the liver, the concentrations of nontargeted ^{99m}Tc-G5-Ac-DTPA decreased with clearance from blood (41.07% ID/g at 2 h to 32.94% ID/g at 6 h), whereas the targeted ^{99m}Tc-G5-Ac-FA-DTPA decreased more slowly (32.02% ID/g at 2 h to 26.17% ID/g at 6 h). In the tumors, the level of ^{99m}Tc-G5-Ac-FA-DTPA increased from 3.10% ID/g at 2 h to 6.78% ID/g at 6 h. The nontargeted ^{99m}Tc-G5-Ac-DTPA also increased slightly from 2.81% ID/g at 2 h to 4.38% ID/g at 6 h.

Micro-SPECT imaging confirmed the predominant uptake of ^{99m}Tc-G5-Ac-FA-DTPA in the FR-positive tumors, liver, and kidneys in the KB tumor–bearing mice (n = 2/time point) (1, 2). Comparison among ^{99m}Tc-G5-Ac-pegFA-DTPA, ^{99m}Tc-G5-Ac-FA-DTPA, and ^{99m}Tc-G5-Ac-DTPA showed that PEGylation of the PAMAM dendrimer-FA

conjugate improves the tumor targeting and may be used as a targeted delivery system for imaging labels and therapeutic drugs (1, 2).

Other Non-Primate Mammals

[PubMed]

No references are currently available.

Non-Human Primates

[PubMed]

No references are currently available.

Human Studies

[PubMed]

No references are currently available.

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