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Radiosynthesis and micro-SPECT imaging of ^{99m}Tc-dendrimer poly(amido)-amine folic acid conjugate

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ABSTRACT

Acetylated (Ac) dendrimer poly(amido)-amine (PAMAM) generation 5 (G5) reacted with folic acid (FA), followed by reacting with 2-(*p*-isothiocyanatobenzyl)-6-methyl-diethylenetria minepentaacetic acid (1B4M DTPA) to form the conjugate of Ac-G5-FA-1B4M DTPA which was further radiolabeled with ^{99m}Tc. The radiochemical yield is up to 98.9% with excellent in vitro/in vivo stability, rapid blood clearance and certain tumor accumulation which was further confirmed by micro-SPECT imaging study.

Drug targeting is critical for effective cancer chemotherapy. Many scientists have explored novel methods for delivering drugs selectively to pathologic cells, thereby avoiding the collateral damage that accompanies their uptake by healthy cells. Recently folic acid (FA) has emerged as an optimal targeting ligand for selective delivery of attached imaging and therapeutic agents to cancer tissues. Folic acid has high affinity to the folate receptor ($K_d = 10^{-10}$ M),[1,2] even after conjugation to its therapeutic/diagnostic cargo. The limited distribution of its receptor (FR) in normal tissues and over-expressed in cancer cells made folic acid relatively satisfactory targeting ligand.[3] Dendrimers represent a unique class of nanostructures, playing an important role in the field of nanobiotechnology.[4–12] Dendrimers are synthesized from branched monomer units in a step-wise manner, thus it is possible to precisely control their molecular properties by choosing different building/branching units and surface functional groups.[13–15] Poly(amidoamine) (PAMAM) dendrimers are the most extensively studied dendrimers.[16] The large numbers of surface functional groups on dendrimer's outer shell can be modified or conjugated with a variety of interesting guest molecules. Over the last several years, increasing interest has been attracted to the application of dendrimers as targeting carriers in cancer therapy and MR imaging.[17–20] Recent studies have demonstrated that the conjugation of special targeting moieties with dendrimers labeled with fluorescein can lead to preferential distribution of the cargo in the targeted tumor cells.[20–29] However the fluorescein labeled dendrimer conjugation is very difficult to be detected in live image study. So radiolabeled dendrimer conjugates will find wide range of applications in SPECT (Single Photon Emission Computed Tomography) or PET (Positron Emission Computed Tomography) image. Monoclonal antibodies with radiolabeled have been prepared by attachment of PAMAM dendrimers loaded with ^{111}In or ^{153}Gd or ^{125}I complexes.[30–33] Although the specificity is better, but the means of chemical synthesis of the complex is a big problem. The nuclear properties of ^{99m}Tc [$T_{1/2}$ 6.01 h; and 140 KeV gamma emissions; low dose burden to patients, and the widespread availability of low cost ^{99}Mo/^{99m}Tc-generators] would make it an ideal SPECT imaging radioisotopes. Herein we report our preliminary result on the radioactive synthesis of ^{99m}Tc-PAMAM-FA conjugates, in vitro/in vivo stability, biodistribution and micro-SPECT image in KB tumor-bearing nude mice.

To increase the solubility and decrease the non-specific cellular uptake, the primary amine on the surface of PAMAM dendrimer were partially converted to acetamide moieties in the presence of acetic anhydride and triethylamine.[23] The degree of acetylation was measured by ^{1}H NMR. We observed a little difference with the reported method probably due to reaction conditions and the effects of temperature. ^{1}H NMR spectrum of the acetylated dendrimer showed the proton signal at d 2.35 ppm, which corresponded to the methylene protons of –CH_2C(O)– in dendrimer PAMAM G5. The specific signal at d 1.93 ppm corresponded to the

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methyl protons of induced acetyl groups. The integration ratio of these two kinds of proton signals in the acetylated dendrimer suggested that average of 77 acetyl groups are present on the surface of each G5 PAMAM dendrimer (Ac77-G5). Folic acid was used in this study which is a 'Trojan horse'[34] for targeting of FR-positive tumors. Conjugation of FA to the partially acetylated dendrimers was carried out via condensation between the γ-carboxyl group of FA and the primary amine of the dendrimer. The active ester of FA, formed by reaction with EDC in DMSO–DMF (1:3), was added dropwise to a solution of DI water containing G5-Ac (77) and vigorously stirred for three days to allow the reaction of FA to the G5-Ac (77) completes.[22,23] In this reaction the γ-carboxylic group possesses a higher reactivity during carbodiimide-mediated coupling to amino groups as compared to the α-carboxyl group.[13] When the γ-carboxylic group on FA is used for conjugation to the dendrimer, FA retains a strong affinity toward its receptor, allowing the FA moiety of the conjugate to retain its ability to act as a targeting agent. The conjugate was purified by membrane filtration and dialysis. Its number of FA in one dendrimer G5-Ac (77) was determined by ^{1}H NMR. For detecting the conjugates in live animal we employed ^{99m}Tc as radioactive nuclide which is commercially available and easy to coordinate with bifunctional chelating agent DTPA. The partially acetylated dendrimer was reacted with 1B4M DTPA whereas isothiocyanates is active enough to react very easily

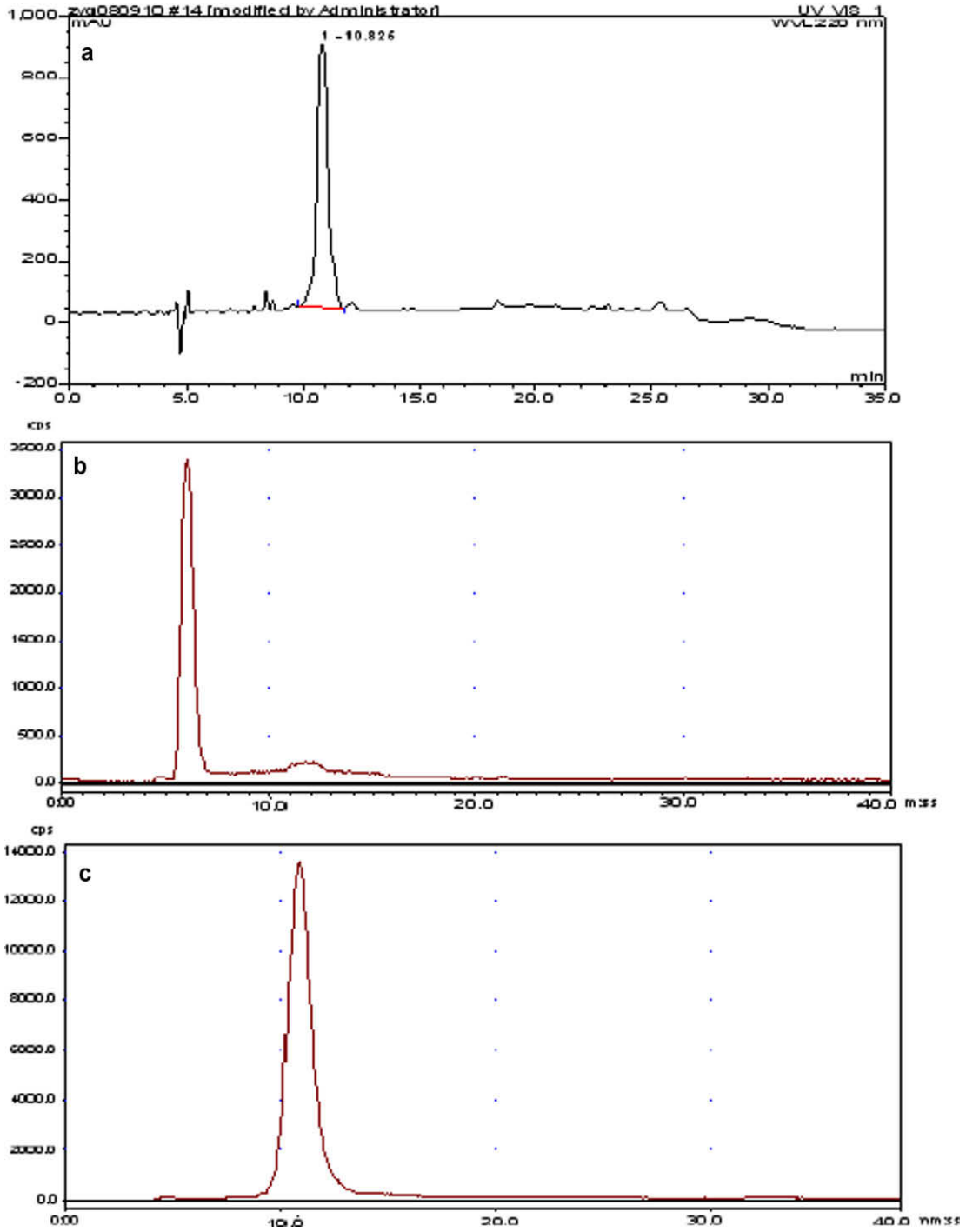


Figure 1. High performance liquid chromatography (HPLC) analyses of the complex. (a) G5-Ac-FA-DTPA(UV–vis detector), (b) $^{99m}TcO_4^+$ (radioactivity γ detector), (c) ^{99m}Tc-G5-Ac-FA-DTPA (radioactivity γ detector).

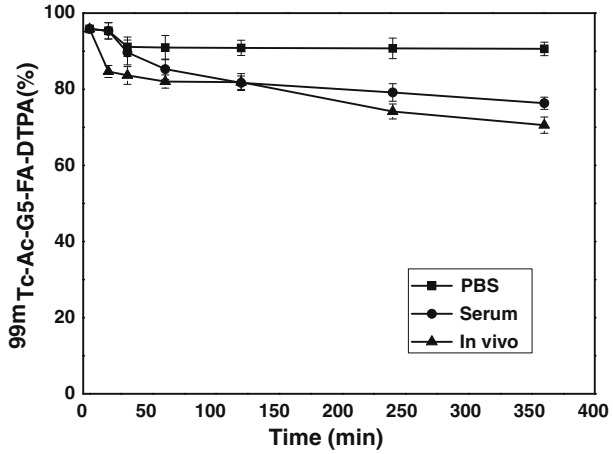


Figure 2. In vitro (PBS, serum) and in vivo stability of the conjugate ^{99m}Tc-G5-Ac-FA-1B4M DTPA.

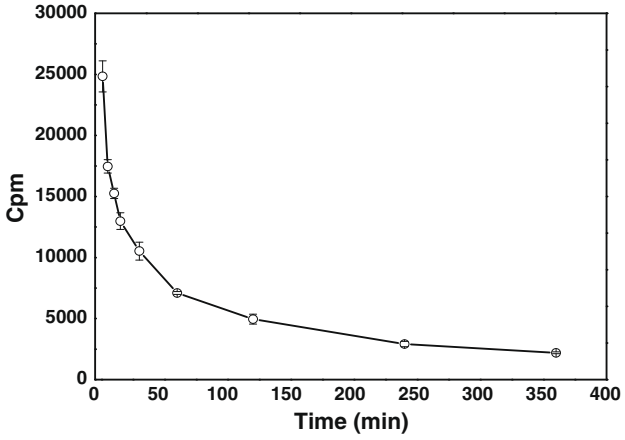


Figure 3. Blood clearance of ^{99m}Tc-F5-Ac-FA-DTPA in normal mice (n = 3).

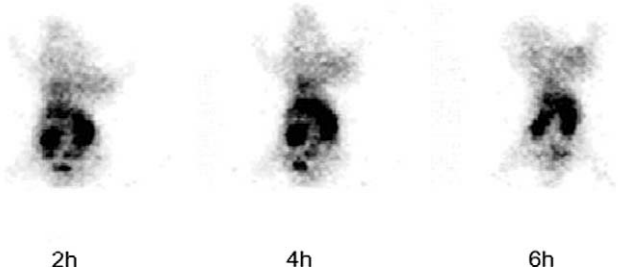


Figure 5. SPECT image of KB-bearing nude mice at 2 h, 4 h and 6 h.

with terminated primary amine,[35] the degree of functionalization can be controlled by stoichiometric control of reagents ratio (Scheme 1).

With G5-Ac-FA-DTPA in hand, we tried to use stannous chloride to reduce $^{99m}TcO_4^-$ to ^{99m}Tc (mixture of +3 and +4) for labeling the conjugate. To our pleasure under optimized conditions ^{99m}Tc labeled G5-Ac-FA-DTPA gave excellent radiochemical yield (98.9%) which can be used directly without further purification (Fig. 1). A high-performance liquid chromatograph, equipped with a radioactivity γ detector, was used to monitor the conversion of G5-Ac-FA-DTPA to ^{99m}Tc-G5-Ac-FA-DTPA. The G5-Ac-FA-DTPA had a retention time of 10.826 min, the $^{99m}TcO_4^+$ had a retention time of 6.005 min, and the radioactive technetium labeled ^{99m}Tc-G5-Ac-FA-DTPA eluted at 11.032 min. Both $^{99m}TcO_4^+$ and ^{99m}Tc-G5-Ac-FA-DTPA were monitored by a gamma detector.

The in vitro stability in PBS and new-born calf serum was studied, results were shown in Figure 2. The radioactive conjugates for all of ^{99m}Tc-Ac-G5-FA-1B4M DTPA keeps excellent in vitro stability in PBS and new-born calf serum at 37 °C within 6 h, more than 90% and 78% conjugate still keeps the original structure, respectively (Fig. 2). And in vivo more than 70% of ^{99m}Tc-Ac-G5-FA-1B4M DTPA conjugate keeps in good stability within 6 h in blood of normal mice (Fig. 2). We next evaluated the pharmacokinetic blood clearance of the ^{99m}Tc-Ac-G5-FA-1B4M DTPA formulation. The blood clearance curve for ^{99m}Tc-F5-Ac-FA-DTPA in normal mice was

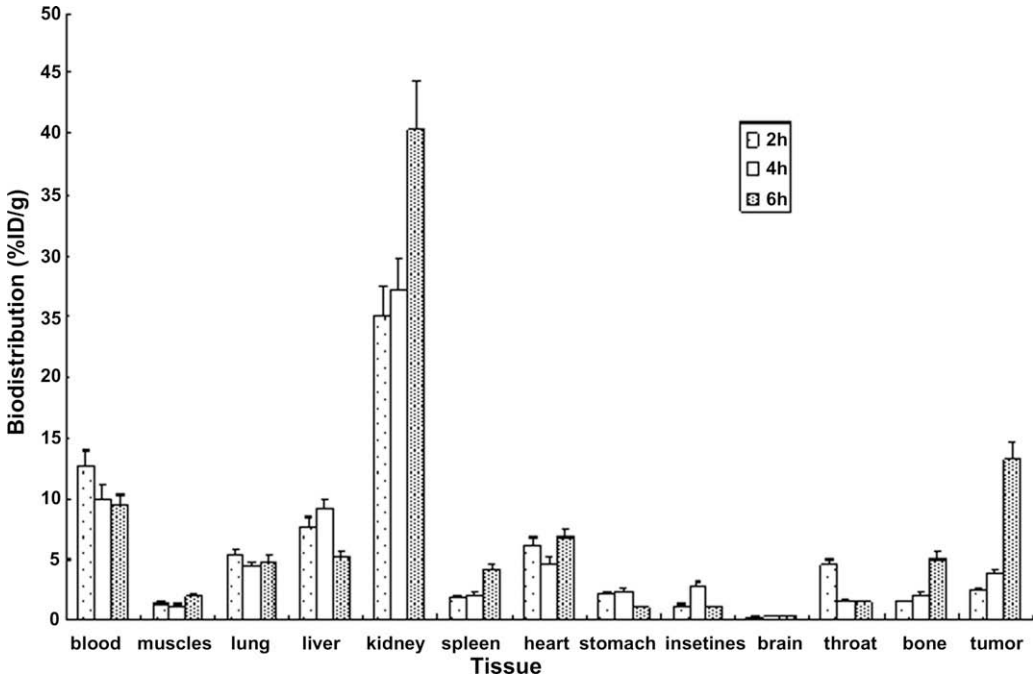
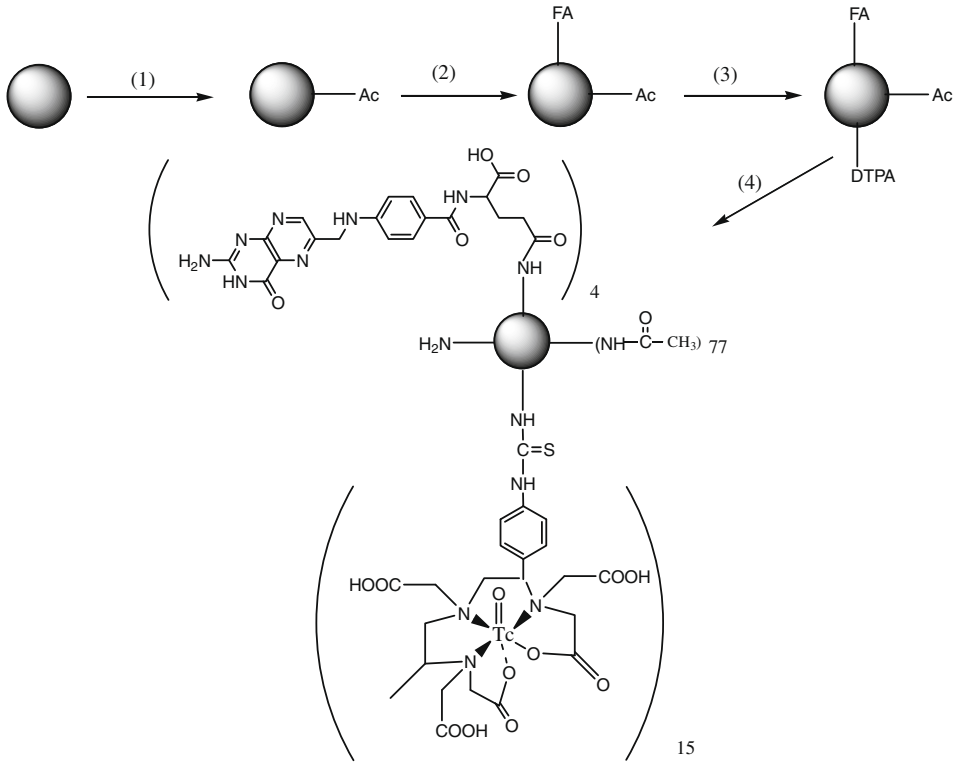


Figure 4. Biodistribution of ^{99m}Tc-G5-Ac-FA-DTPA in KB-bearing nude mice.



Scheme 1. Synthesis of ^{99m}Tc-Ac-G5-FA-1B4M-DTPA. Reagents and conditions: (1) Et_3N, acetic anhydride, CH_3OH, 18 h. (2) EDC·HCl, DMF/DMSO, 3 d (3) pH 8–10, 2 d. (4) Sn^{2+}, TcO_4^-, 80 °C, 30 min.

shown in Figure 3. The concentration–time curve was fitted as a pharmacokinetic two-compartment model and expressed as shown in the equation below. A rapid decrease was observed, followed by a very slow clearance after 30 min. The plasma half life ($t_{1/2}$) of the tracer in the blood of normal mice was as follows: $\alpha_{t1/2}$ = 13.5 min and $\beta_{t1/2}$ = 145.9 min. These data indicates that the dendrimer folic acid conjugate was rapidly cleared from blood. Partition coefficients of the radiolabeled conjugates were determined by the ratio between *n*-octanol and water. The partition ratio of the conjugate was $P = 0.0282$ ($\log P = -1.55$). The property of excellent water solubility greatly improves the radiolabeled yield.

The in vivo bio-distribution of ^{99m}Tc-Ac-G5-FA-1B4M DTPA in KB-bearing nude mice was assessed by injecting BALB/c nude mice. The amount of radioactivity in the tumor was increased with the time while decreased in the blood (Fig. 4). At 6 h, the uptake of ^{99m}Tc-Ac-G5-FA-1B4M DTPA by liver (9.48 ± 0.51% ID/g), heart (6.88 ± 0.47% ID/g) was expected considering the character of the PAMAM. Stomach, pancreas, muscle, throat and brain showed minimal uptake of the conjugate. ^{99m}Tc-Ac-G5-FA-1B4M DTPA showed some accumulation in tumor and kidneys. The higher uptake occurred in the kidneys, consistent with the known presence of folate receptors in the proximal tubules of the kidneys,[36–39] and also may be concerned with the molecular weight of ^{99m}Tc-Ac-G5-FA-1B4M DTPA. At 6 h postinjection, the tumor uptake of ^{99m}Tc-Ac-G5-FA-1B4M DTPA was 13.34 ± 1.36% ID/g (n = 3).

The predominant uptake of ^{99m}Tc-G5-Ac-FA-DTPA by the FR-positive tumors and kidneys was further confirmed by micro-SPECT image study. As shown in Figure 5, ventral image was taken for a mouse from 2 h to 6 h postinjection of 18.5 MBq dose of ^{99m}Tc-G5-Ac-FA-DTPA which the γ radiation distinctively localizes in the kidney and the KB tumor cells (axilla region). Less appreciable radiotracer was observed in brain and muscle. The Micro-SPECT imaging further confirmed the conjugate of ^{99m}Tc-G5-Ac-FA-1B4M DTPA concentrated in tumor as time increased.

In summary, ^{99m}Tc radiolabeled dendrimer PAMAM-G5-folic acid conjugate was synthesized successfully with excellent in vitro/in vivo stability and rapid clearance from blood. Biodistribution study of KB tumor-bearing nude mice showed certain accumulation was observed in tumor-bearing nude mice which was further confirmed by micro-SPECT imaging study.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.12.075.

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