An Improved Synthesis and Preliminary Biodistribution Study of a Technetium-99m-labeled 2-amino-2-deoxy(thioacetyl)-D-glucose Complex ([99mTc]-TA-DG) As a Tumor Imaging Agent

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ABSTRACT

Introduction: This report describes the synthesis of 2-Amino-2-deoxy(S-benzoylthioacetyl)-D-glucose (S-Bz-TA-DG), radiolabeled with [99mTc(CO)₃(OH₂)₃]⁺ complex with a procedure including deprotection of the benzoyl group, characterization by HPLC using a C18 reverse phase column and preliminary biodistribution study in normal mice.

Methods: [99mTc(CO)₃(H₂O)₃]⁺ complex was used to label TA-DG with 99mTc. This complex was prepared using up to 46 mCi of Na ⁹⁹mTcO₄ in 1mL saline. The radiochemical purity (>95%) was determined by TLC in normal saline solution as the mobile phase. Radio-HPLC analysis of [99mTc]-(TA-DG) at pH=9.5-10, revealed that labeling with ⁹⁹mTc resulted in the formation of three radiochemical species (Na ⁹⁹mTcO₄ with tₑ=5.7 min, [⁹⁹mTc(CO)₃(H₂O)₃]⁺ complex with tₑ=27.5 min and [⁹⁹mTc]-(TA-DG) [yield >85%] with tₑ=8.2 min) with different HPLC-profiles.

Results: The biodistribution of the [⁹⁹mTc]-(TA-DG) complex was studied in normal mice (body mass 25–35 g) at 30 min and 1 h post-injection, according to a published procedure. This complex showed negligible brain uptake (0.13%±0.03 ID) at 30 min post-injection; an efficient clearance from the blood, a rapid excretion to the urine and a low retention in the liver and kidneys.

Conclusion: Nonfunctionalized carbohydrate compounds such as glucose are generally weak ligands for chelating with ⁹⁹mTc. Therefore, functionalization with an external chelating group or the insertion of some functional groups is essential to obtain strong metal-binding compounds. On the basis of our results, it seems that [⁹⁹mTc]-(TA-DG) has not most of the favorable properties as an imaging agent for brain tumors.

Key words: ⁹⁹mTc, Glucosamine derivatives, ⁹⁹mTc-tricarbonyl complex, 2-amino-2-deoxy (thioacetyl)-D-glucose


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Introduction

Carbohydrates are interesting building blocks in organic chemistry and drug development due to their involvement in various biological systems, water solubility, and optical activity (1). Accurate and early non-invasive detection of malignant disease is an important factor in the treatment and prognosis of a patient with cancer. Improvements in tumor radionuclide imaging depend on the development of more tumor-specific radiopharmaceuticals (2). Fluorine-18 (18F) fluorodeoxyglucose (FDG) has been used to measure normal tissue and tumor glucose utilization rates. Although metabolic tumor imaging with [18F]-FDG has been studied for more than two decades, the use of this examination in clinical practice is still limited by factors such as difficult access, limited availability, and high cost. In addition, positron emission tomography (PET) radio-synthesis must be performed rapidly because the half-life of 18F is only 109 min. Thus, it would be very desirable to develop less costly imaging agents based on γ-emitter isotopes, especially for developing countries, where single photon emission computed tomography (SPECT) is still dominant. Technetium-99m (99mTc) has been mostly used for radiopharmaceuticals labeling due to its suitable physical and chemical characteristics and inexpensive isotope cost (3). Organometallic metal cores often exhibit advantages in terms of kinetic inertness, stability, and size and thus could lead to the development of more efficient and stable compounds compared to classical inorganic complexes (4). We elected to use the versatile low valent fac-[99mTc(I)-(CO)3] core, which its chemistry has recently been pioneered by Alberto and co-workers. The facially coordinated carbonyl ligands stabilize the Tc +1 oxidation state, obviating the elaborate, often coordinated carbonyl ligands stabilize the Tc +1 oxidation state, obviating the elaborate, often macrocyclic, polydentate structures required to stabilize other intermediate oxidation states of Tc. In neutral complexes with simple N,O donors the fac-[99mTc(I)-(CO)3] core possesses intermediate lipophilicity, an advantage in living systems (5). Lots of 99mTc-labeled glucose derivatives have been synthetized in order to develop one subrogate in SPECT for [18F]-FDG in PET recently. Developed by Yang, 99mTc-labeled ethylenedicysteine-deoxyglucose (ECDG) showed similarities with [18F]-FDG in tumor uptake. This suggests that there is feasibility for 99mTc-labeled deoxyglucose as a metabolic tumor imaging agent. However, [99mTc]-ECDG still has some drawbacks such as slow cleanup from the blood, which would cause high background activity; and large molecular weight, which would limit its penetration through blood–brain barrier (BBB). Thus, it would be desirable to develop a smaller 99mTc-based deoxyglucose derivative with rapid blood clearance and still maintaining its high tumor uptake (3).

Methods

All chemicals were purchased from Fluka and Merck companies and used without further purification. Carbon monoxide was obtained in the form of refillable canisters (0.5 L) from M/s Alchemie Gases & Chemicals, Atomic Energy Organization of Iran. 99mTcO4− was eluted from an in-house 99Mo/99mTc column generator using normal saline. High Performance Liquid Chromatography analyses were performed on a JASCO 880-PU HPLC(Tokyo, Japan) equipped with a Raytest-Gabi gamma ray detector. A Polygosil 5μm RP-C18 analytical column (reverse phase) with dimensions of 250×4.6 mm was used. The NMR spectra were measured on a 500 MHz, and IR spectra were recorded on a FT-IR-BOMEM- MB-Series. All radioactivity measurements were carried out using NaI(Tl) scintillation counter.

Synthesis of S-benzoylthioglycolic acid (1)

Synthesis of (1) was done according to the previously reported method (6). Sodium hydroxide (8.8 g, 220 mmol) and thioglycolic acid (9.2 g, 100 mmol) were dissolved in a mixture of toluene (75 mL) and water (75 mL). The mixture cooled in an ice-acetone bath to about −5−0 °C. Benzoyl chloride (14.05 g, 100 mmol) was added over 30 minutes, and stirring was continued for 30 minutes at −5−0 °C and another 30 minutes at room temperature. The organic layer was separated, washed with water (4×50 mL) and the combined aqueous phases were acidified to pH 1.5 by hydrochloric acid. The precipitated product was...
filtered and dried. Recrystallization from ethyl acetate gave 16.1 g (83.0%) of product as colorless crystals with a melting point of 99–101 °C (literature value = 102–103 °C). IR (KBr), ν (cm⁻¹): 1712 (-COOH), 1660 (-CO-), 2916 (-CH, stretching). ¹H NMR (CDCl₃), δ (ppm): 3.97 (s, 2H), 7.49-8.02 (m, 5H, aromatic); ¹³C NMR (CDCl₃), δ (ppm): 3.6 (d, 1H), 6.01-6.5 (m, 5H, aromatic).

Synthesis of S-benzoylacetyl Chloride (2)

Synthesis of (2) was done according to the previously reported method (7). A solution of S-benzoylthioglycolic acid (1 g, 5.1 mmol, 0.26 equiv) in dichloromethane (5 mL) was charged sequentially with oxalyl chloride (1.05 mL, 12.1 mmol) and N,N-dimethylformamide (10 µL, 0.13 mmol, 0.005 equiv), and the mixture was stirred under argon for 1 h at room temperature. The color of the reaction mixture changed from colorless to yellow. The solvent was evaporated under reduced pressure. The excess of oxalyl chloride was removed by evaporating with a vacuum pump. The obtained yellow solid (m.p = 72-75 °C) was used for the next step. IR (KBr), ν (cm⁻¹): 2987 (-CH, stretching), 1815 (-COCl), 1660 (-COS-), 2916 (-CH, stretching). ¹H NMR (CDCl₃), δ (ppm): 4.4 (s, 2H), 7.5-8.03 (m, 5H, aromatic); ¹³C NMR (CDCl₃), δ (ppm): 42.3, 128.1, 129.3, 134.4, 136.3, 175.4, 190.5.

Synthesis of 2-Amino-2-Deoxy(S-Benzoylthioacetyl)-D-Glucose (3)

Synthesis of (3) was done according to the previously reported method (8). S-benzoylthioacetyl chloride (1.0 g, 4.66 mmol) was dissolved in dichloromethane (2 mL). This solution was added drop wise to a solution of glucosamine hydrochloride (0.67 g, 3.11 mmol) and sodium hydrogen carbonate (0.52 g, 6.22 mmol) in 10 mL of water. The mixture was stirred for 1 hour at 60 °C, and then the precipitated product was filtered and dried. The resulting precipitate was washed with cold water (20 mL) and diethyl ether (15 mL). The solid was dried in a desiccators containing silica gel under vacuum. The yield of 3 was 78.0%; m.p = 193–195 °C (dec.). IR (KBr), ν (cm⁻¹): 3300-3500 (-OH and -NH, stretching), 2916 (-CH, stretching), 1660.5 (-CO- and -CON-), 1557 (-NH); ¹H NMR (CD₃OD), δ (ppm): 1.8-1.9 (m, 2H), 2.19-2.45 (m, 9H), 3.6 (d, 1H), 6.01-6.5 (m, 5H, aromatic); ¹³C NMR (CD₃OD), δ (ppm): 31.1, 53.8, 60.3, 69.9, 70.3, 70.6, 89.9, 125.8, 127.5, 132.5, 135.3, 168.4, 189.7.

Preparation of ⁹⁹mTc tricarbonyl precursor, [⁹⁹mTc(OH₂)₃(CO)₃]⁺, (4)

Synthesis of (4) was done according to the previously reported method (9). The ⁹⁹mTc tricarbonyl precursor 4 was prepared using a modification of the procedure described by Alberto et al. A 10 mL vial containing Na₂CO₃ (5 mg), NaBH₄ (4 mg) and sodium potassium tartarate (10 mg) was capped with a rubber stopper and then flushed with a stream of CO gas (99.5%) at room temperature for 10 min. One mL of sodium pertechnetate (Na⁹⁹mTcO₄) with up to 20-100 mCi was added by a syringe and then heated to 75 °C for 30 min. After rapid cooling down to room temperature, 0.3 mL of 0.1M HCl was added to decrease the pH (pH=9.5-10). The yield was >98% which was determined by HPLC.

Preparation of ⁹⁹mTc(I) tricarbonyl (TA-DG) complex (5)

Synthesis of (5) was done according to the previously reported method (4). ⁹⁹mTc(I) tricarbonyl precursor 4 (300 µL) was added into 1 mL of S-Bz-TA-DG solution (0.0010M in phosphate buffer 0.05M with pH=9.5-10) and then the reaction mixture was heated 75 °C for 30 min. After cooling it to room temperature, ⁹⁹mTc(I) tricarbonyl (TA-DG) was characterized. Analytical data for yield: >85% determined by means of HPLC with solvents consisted of 0.1% trifluoracetic acid in water (solvent A) and acetonitrile (solvent B).

Results and Discussion

Chemistry/synthesis

The chemistry necessary for the synthesis of the S-Bz-TA-DG was mainly based on organic chemistry techniques. Due to the poor chemical stability of the thiol-group, S-Bz-TA-DG were synthesized as the thiol-protected precursors, the S-benzoyl protecting group being split off during chelation with ⁹⁹mTc at elevated temperature and high pH (10). [⁹⁹mTc(CO)₃(H₂O)₃]⁺ has proved to be an excellent agent for labeling different kind of ligands. It can be formed in high yield directly from generator eluted pertechnetate in aqueous solution. As three coordinated water are labile, they could be exchanged readily with a variety of mono-, bi- and tridentate ligands forming complexes. The major advantage of using the carbonyl precursor is that high specific activity labeling of biomolecules can be obtained (11).

HPLC characterization

Characterization of the [⁹⁹mTc(CO)₃(H₂O)₃]⁺ and ⁹⁹mTc(TA-DG) complex was carried out by HPLC using a C18 reverse phase column. HPLC solvents consisted of 0.1% trifluoracetic acid in water (solvent A) and acetonitrile (solvent B).
Samples were analyzed with a linear gradient method (100% solvent A to 100% solvent B over 30 min). The test solution (20 mL) was injected into the column and the elution was monitored by observing the radioactivity profile. The flow rate was maintained at 1 mL/min.

**Stability studies**

The $^{99m}$Tc-(TA-DG) complex (0.5 mL) was incubated with human serum (0.4 mL) at 37 °C for 7 h. The stability of this complex was studied by HPLC analysis and monitoring the elution profile.

**Animal biodistribution studies**

Biodistribution experiments of injected radiolabeled sugar was performed in normal mice (25–35 g) as previously described (12). Each animal was cited in specific container. After the injection of 0.1-0.3 mL (5mCi) of the complex into a lateral tail vein, the blood, lung, liver, spleen, kidney, stomach, intestine, brain and heart were harvested, weighted, counted and the %ID/g were determined.

**Labeling with $^{99m}$Tc**

$^{99m}$Tc(tricarbonyl precursor 4, $[^{99m}$Tc(CO)$_3$(H$_2$O)$_3$]$^+$, was successfully prepared with 98% radiolabeling using a modification of the procedure described by Alberto et al. The complex is stable (>62%) for approximately 3 h. After this time, decomposition of the complex was observed. The $^{99m}$Tc complex of the TA-DG was obtained by addition of $[^{99m}$Tc(CO)$_3$(H$_2$O)$_3$]$^+$ at alkaline pH values (pH 9.5-10). Since the deprotonation of the amide nitrogens and removal of the S-protecting group in MAG$_3$-like agents is facilitated under alkaline conditions, a relatively high pH for the labeling reaction mixtures was preferred in order to maximize the labeling yield as well as to minimize the formation of side products. Radio-HPLC analysis of $[^{99m}$Tc](TA-DG) at pH=9.5-10, revealed that labelling with $^{99m}$Tc resulted in the formation of three radiochemical species (Na $^{99m}$TcO$_4$ with t$_R$=5.7 min, $[^{99m}$Tc(CO)$_3$(H$_2$O)$_3$]$^+$ complex with t$_R$=27.5 min and $[^{99m}$Tc]-TA-DG, t$_R$ = 8.2 min.

**Biodistribution in mice**

The animals were housed at The Animal Center of Atomic Energy Organization of Iran. The biodistribution of the $[^{99m}$Tc]- (TA-DG) complex was studied in normal mice (body mass 25–35 g) at 30
min and 1 h post-injection, according to a published procedure (12). The results of the biodistribution study of $[^{99m}Tc]$-(TA-DG) is summarized in Table 1.

**Table 1. Biodistribution of $[^{99m}Tc]$-TA-DG in normal mice**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>30min</th>
<th>1 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>0.13±0.03</td>
<td>0.07±0.05</td>
</tr>
<tr>
<td>Kidney</td>
<td>8.80±0.61</td>
<td>10.81±5.03</td>
</tr>
<tr>
<td>Lung</td>
<td>2.61±0.32</td>
<td>1.68±0.15</td>
</tr>
<tr>
<td>Heart</td>
<td>0.91±0.16</td>
<td>0.52±0.10</td>
</tr>
<tr>
<td>Spleen</td>
<td>1.97±0.18</td>
<td>1.36±1.29</td>
</tr>
<tr>
<td>Stomach</td>
<td>3.62±0.08</td>
<td>3.08±0.21</td>
</tr>
<tr>
<td>intestine</td>
<td>1.98±0.71</td>
<td>3.07±1.32</td>
</tr>
<tr>
<td>Liver</td>
<td>5.80±1.01</td>
<td>7.19±1.61</td>
</tr>
<tr>
<td>Blood</td>
<td>3.24±0.43</td>
<td>1.52±0.61</td>
</tr>
</tbody>
</table>

* All data are the mean percentage (n = 3) of the injected dose of $[^{99m}Tc]$-TA-DG per gram of wet tissue ± the standard deviation of the mean.

This complex showed negligible brain uptake (0.13%±0.03 ID) at 30 min post-injection, an efficient clearance from the blood, a rapid excretion to the urine and a low retention in the liver and kidneys. On the basis of these results, it seems that $[^{99m}Tc]$-(TA-DG) has not most of the favorable properties as an imaging agent for brain tumors. The benzoyl protected precursor of thioacetylglicosamine was synthesized following the synthetic route outlined in Scheme 1. In the course of synthesis of S-benzylothioglucolic acid 1, the time of adding benzoyl chloride must be over 30 minutes. The rate of adjusting pH should not be fast otherwise the product yield is lower. S-benzylothioacetyl chloride 2 is a suitable product, which can be coupled with a variety of sugars. This can offer the possibility of synthesizing modified NS ligands in a simple one-step reaction. Compounds 1 and 2 were easily prepared in good yield. The product was confirmed by melting point, IR and NMR. The overall yield of S-Bz-TA-DG is 78.0%. In our studies, the optimal pH value for deprotection of S-benzoyl group was between 9.5-10, and the reaction time was 30 minutes. We did not obtain $[^{99m}Tc]$-(TA-DG) at neutral pH value or at pH values above 10, because the thioester bond of S-Bz-TA-DG is hardly disassociated in aqueous solution of pH neutral, and the glucosamine was destroyed in aqueous solution with pH values above 10.

**Conclusion**

Nonfunctionalized carbohydrate compounds such as glucose are generally weak ligands for chelating with $^{99m}$Tc. Therefore, functionalization with an external chelating group or the insertion of some functional groups is essential to obtain strong metal-binding compounds. In our studies, $[^{99m}Tc]$-(TA-DG) was synthesized by procedure including deprotection of the benzoyl group. The labeling procedure took place at higher pH. Low molecular weight accelerated clearance from blood, and different linkers and chelate cores changed excretion path. On the basis of our results, it seems that $[^{99m}Tc]$-(TA-DG) has not most of the favourable properties as an imaging agent for brain tumors.

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