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Technetium glucose complexes as potential cancer imaging agents

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ABSTRACT

GLUT's (facilitative glucose transporters) over-expression in tumor cells has allowed the detection of several cancer types, using a glucose analogue (^{18}F-FDG) with PET images, worldwide. New glucose analogs radiolabeled with ^{99m}Tc could be a less-expensive and more accessible alternative for diagnosis using SPECT imaging. D-Glucose (^{99m}Tc-IDAG) and 2-D-deoxyglucose (^{99m}Tc-AADG) organometallic complexes were proposed and studied as potential ^{18}F-FDG surrogates. The glucose complexes were prepared and evaluated as potential cancer imaging agents, in a melanoma tumor model. Iminodiacetic acid (IDA) and aminoacetate (AA) moieties were chosen as chelating system for radiolabeling with ^{99m}Tc. Tumor uptake of the formed complexes was evaluated in B16 murine cell line in vitro and in vivo in melanoma bearing C57BL/6 mice. In vitro and in vivo studies were conducted with ^{18}F-FDG in order to compare the uptake of ^{99m}Tc-glucose complexes in the tumor model. IDAG and AADG compounds were synthesized and radiolabeled with $^{99m}TcO_4^-$ to obtain the ^{99m}Tc-IDAG and ^{99m}Tc-AADG complexes in high yield and stability. In vitro cell studies showed maximum uptake at 60 min for complexes, ^{99m}Tc-IDAG and ^{99m}Tc-AADG, with 6% and 2%, respectively. Biodistribution studies showed high tumor uptake one hour post-injection, reaching tumor-to-muscle ratios of 12.1 ± 3.73 and 2.88 ± 1.40 for ^{99m}Tc-IDAG and ^{99m}Tc-AADG, respectively. SPECT and micro-SPECT-CT images acquired after the injection of ^{99m}Tc-IDAG showed accumulation in tumor sites, suggesting that this glucose complex would be a promising candidate for cancer imaging.

Tumor cells are characterized for having an accelerated metabolism and high glucose uptake compared to normal cells. This abnormality is necessary in order to accomplish the fast replication rate of tumor cells, by using energy from glycolysis.[1,2] This abnormal process was called Warburg effect and the major product obtained, even in the presence of oxygen, is lactate.[3,4] Moreover, it has been described that the production of lactate participates in the translocation of more glucose transporters (facilitative transporters or GLUTs) to the outer membrane increasing the uptake of glucose in tumor cells.[5] ^{18}F-FDG-PET is the most common system detection used for cancer imaging in nuclear clinics worldwide and is based on this abnormal phenotype in cancer.[6–8] Although ^{18}F-FDG is widely used for cancer diagnosis, its use is still limited by factors such as difficult access, limited availability and high cost.[9] In this sense, glucose derivatives radiolabeled with γ-emitter isotopes for SPECT are desirable because of its lower cost and availability, especially in developing countries.[10,11] 99m-Technetium is the most used γ-emitter radionuclide in nuclear medicine due to its physical and chemical characteristics ($T_{1/2}$ = 6 h, γ energy = 150 keV) and low cost.[12,13] Several ^{99m}Tc-glucose derivatives have been developed and evaluated in different tumor cells lines in vitro and in vivo, but just one, ethylenedicysteine–deoxyglucose (ECDG, Fig. 1) labeled with ^{99m}Tc, is in Phase II of clinical trials.[14–26] Recently, a deoxy-D-glucose derivative (CPADG) was synthesized and radiolabeled with $^{99m}TcO_4^-$ (^{99m}Tc-CPADG, Fig. 1).[27] This glucose complex showed good in vitro cell uptake (murine sarcoma cells), possibly transported via the glucose transporters. Therefore, further investigation to discover novel ^{99m}Tc labeled glucose analogues for tumor imaging is still necessary.[28] Nonfunctionalized glucose compounds are generally weak ligands for complexing with ^{99m}Tc. Because of this low reactivity, addition of chelating groups is a solution for developing new ^{99m}Tc labeled glucose

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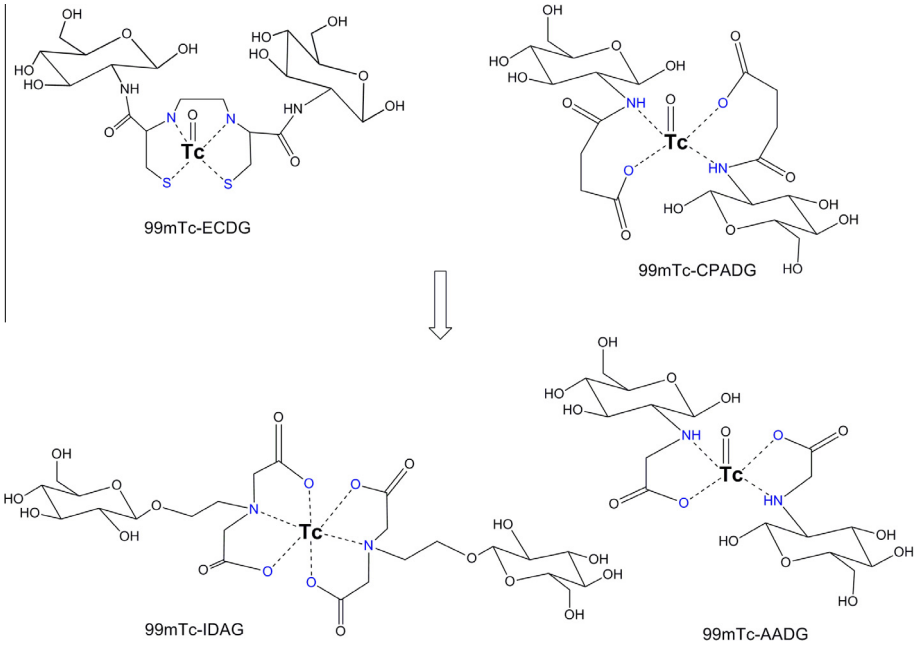
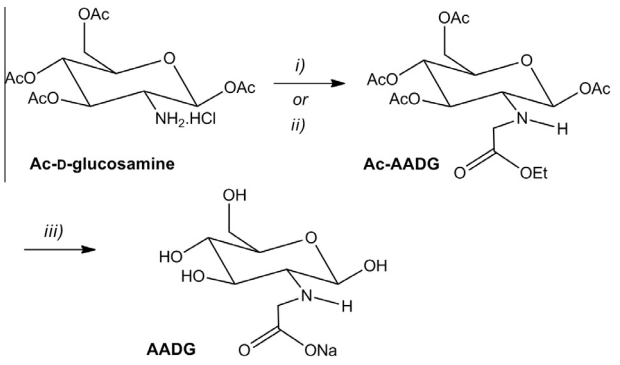


Figure 1. 99m-Technetium glucose complexes.

derivatives as tumor imaging agents. In this context, aminoacetate (AA) or iminodiacetate (IDA) are two common chelators used as bifunctional chelating agents (BFCA) to form ^{99m}Tc complex on the basis of efficient binding of ^{99m}Tc to a nitrogen atom and carboxylic groups.[29–31] The purpose of the present work is to synthesize glucose and 2-deoxyglucose derivatives containing appropriate chelators for labeling with ^{99m}Tc, as cancer imaging agents (Fig. 1). In this sense, AA group was proposed for derivatization at C-2 of 2-deoxy-amino-D-glucose (AADG, Fig. 1). The IDA-glucose derivative (IDAG, Fig. 1) has been previously synthesized by our group. Tumor uptake of the formed complexes was finally evaluated in B16 murine cell line in vitro and in vivo in melanoma bearing C57BL/6 mice. Besides, in vitro and in vivo studies were conducted with ^{18}F-FDG in order to compare the uptake of ^{99m}Tc-glucose complexes in the tumor model proposed.

Synthesis of glucose derivative IDAG was performed as previously described by our group.[22] In order to obtain the 2-glucosamine derivative AADG, different synthetic methodologies were carried out using tetraacetylated glucosamine hydrochloride (Ac-D-glucosamine) as starting material (Scheme 1).



Scheme 1. Reagents and conditions: (i) Ethyl glyoxalate, Acetonitrile/H_2O (3:1), $NaCNBH_3$, 51%; (ii) Ethyl bromoacetate, triethylamine, DMF, 15 h at 100 °C, 10% or Ethyl bromoacetate, triethylamine, DMF, 5 min at 150 °C in microwave 8%; (iii) i-NaOMe, MeOH, ii-NaOH, H_2O, quantitative yield.

Ac-D-glucosamine was prepared from commercially available glucosamine hydrochloride in three reaction steps as described previously.[32]

From Ac-D-glucosamine, two synthetic strategies were carried out to obtain the conjugated compound Ac-AADG, which incorporates the precursor of the aminoacetate ligand. The first one followed previous publication and involved a double reductive amination using an adequate aldehyde.[33] Ethyl glyoxalate was mixed with Ac-D-glucosamine in CH_3CN/H_2O 3:1 and then sodium cyanoborohydride was added. After a 12-h agitation period at room temperature, the reaction yielded only the mono-alkylated product Ac-AADG, without the formation of the di-alkylated product. The second strategy involved an *N*-alkylation of tetra-acetylated D-glucosamine with ethyl bromoacetate in DMF with conventional heating at 100 °C for 15 h. When such temperatures and heating times are needed to complete reactions, microwave heating (MW) is an interesting tool to reduce reaction times (from days and hours to minutes and seconds) and to be able to heat at temperatures higher than the boiling point of reactants or solvents.[34] In this sense, reaction was tested at 150 °C for 5 min in MW, again only product Ac-AADG was obtained. Acetate groups of D-deoxyglucose derivative were removed in sodium methoxide and hydrolysis of ester group with sodium hydroxide was done to obtain the ester salt AADG in excellent yield.

Preparation of ^{99m}Tc-IDAG and ^{99m}Tc-AADG complexes was accomplished through a simple procedure in a similar way as previously described.[23] This procedure is based on the reaction of $[^{99m}TcO_4]^-$ with IDAG or AADG in the presence of stannous chloride as reducing agent to form the final product. Derivative IDAG contained an IDA moiety also present in HIDA like-radiopharmaceuticals.[29] Final complex of the ^{99m}Tc-IDA radiopharmaceuticals is an octahedral structure with two IDA molecules bounded to one technetium atom with oxidation state of +3. Therefore, the expected structure of ^{99m}Tc-IDAG was similar to that shown in Figure 1. A AADG molecule can coordinate to the ^{99m}Tc(V) center with the carboxyl oxygen and one nitrogen atom. Thus, two AADG molecules form bidentate chelates to ^{99m}Tc(V) to obtain a glucose complex (^{99m}Tc-AADG, Fig. 1).[35,36] Radiochemical purity was controlled by paper chromatography; which showed low levels