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A novel D-glucose derivative radiolabeled with technetium-99m: Synthesis, biodistribution studies and scintigraphic images in an experimental model of Ehrlich tumor

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ABSTRACT

A D-glucose-MAG_3 derivative was successfully synthesized and radiolabeled in high labeling yield. Biodistribution studies and scintigraphic images in Ehrlich tumor-bearing mice were performed. This compound showed high accumulation in tumor tissue with high tumor-to-muscle ratio. Thus, D-glucose-MAG_3 could be considered as agent for tumor diagnosis.

Cancer is one of the most common causes of death. Traditionally, the diagnosis was based on anatomical details of the organs under study. But, with the advent of nuclear medicine, it has become possible to identify biochemical changes in the disease even before the presence of anatomical changes can be observed. 'In vivo' functional imaging may aid in the diagnosis and therapy of patients so as to increase their survival rate.[1,2]

Positron emission tomography (PET) with the [^{18}F] 2-fluoro-2-deoxy-D-glucose, ([^{18}F] FDG) is widely used in the diagnosis and monitoring of cancer.[3] The [^{18}F] FDG is an analogue of D-glucose, and its uptake is based on the higher glycolytic enzyme activity of malignant cells compared to normal tissue.[2,4–6]

Although the metabolic imaging of tumors with [^{18}F] FDG has been studied for over two decades, its clinical use is still limited because of some factors such as difficult access, limited availability and high cost. The development of diagnostic agents based on gamma radiation-emitting isotopes for the production of images in conventional scintillation cameras would be of great value.[1,7]

Some studies have been conducted in an attempt to develop new radiopharmaceuticals involving technetium-99m (^{99m}Tc) complexes with analogues of D-glucose for the diagnosis of cancer.[1,4,8–10] The ^{99m}Tc is the most widely used radionuclide in nuclear medicine because it presents appropriate physical and chemical properties such as a physical half-life of 6.01 h and low energy gamma emission (140 keV), in addition to a relatively low cost when compared with [^{18}F] fluoride.[1,11–14]

An experimental model that can be used to search for tumor is based on the implantation of Ehrlich tumor cells in the hind thigh of mice. The tumor is of the rapidly developing Ehrlich cells from easily transplantable mammary adenocarcinoma from female mice. Such cells have been employed as a model in the study of various products used to treatment and diagnoses.[15,16]

Carbohydrates such as D-glucose are generally weak ligands for complexation with ^{99m}Tc. Thus, the functionalization of the sugar with an external chelating group is crucial for obtaining a compound that effectively binds the metal.[17]

The mercaptoacetylglycylglycylglycine is a complexing agent of proven effectiveness for ^{99m}Tc, and it is used as a renal tubular radiotracer.[18,19] It has a carboxylic acid group that can couple with compounds containing amino groups.

The purpose of this study was performed the biodistribution studies and scintigraphic images of ^{99m}Tc-MAG_3 and the new complex ^{99m}Tc-MAG_3-glucose for evaluating the feasibility of the ^{99m}Tc-labeled glucose derivative as candidate for tumor diagnosis agent.

The D-glucose-MAG_3 derivative **6** was synthesized according to the procedure outlined in Scheme 1.

The 4-nitrophenyl β-D-glucopyranoside **1** was reduced to 4-aminophenyl β-D-glucopyranosíde **2** using catalytic hydrogenation.

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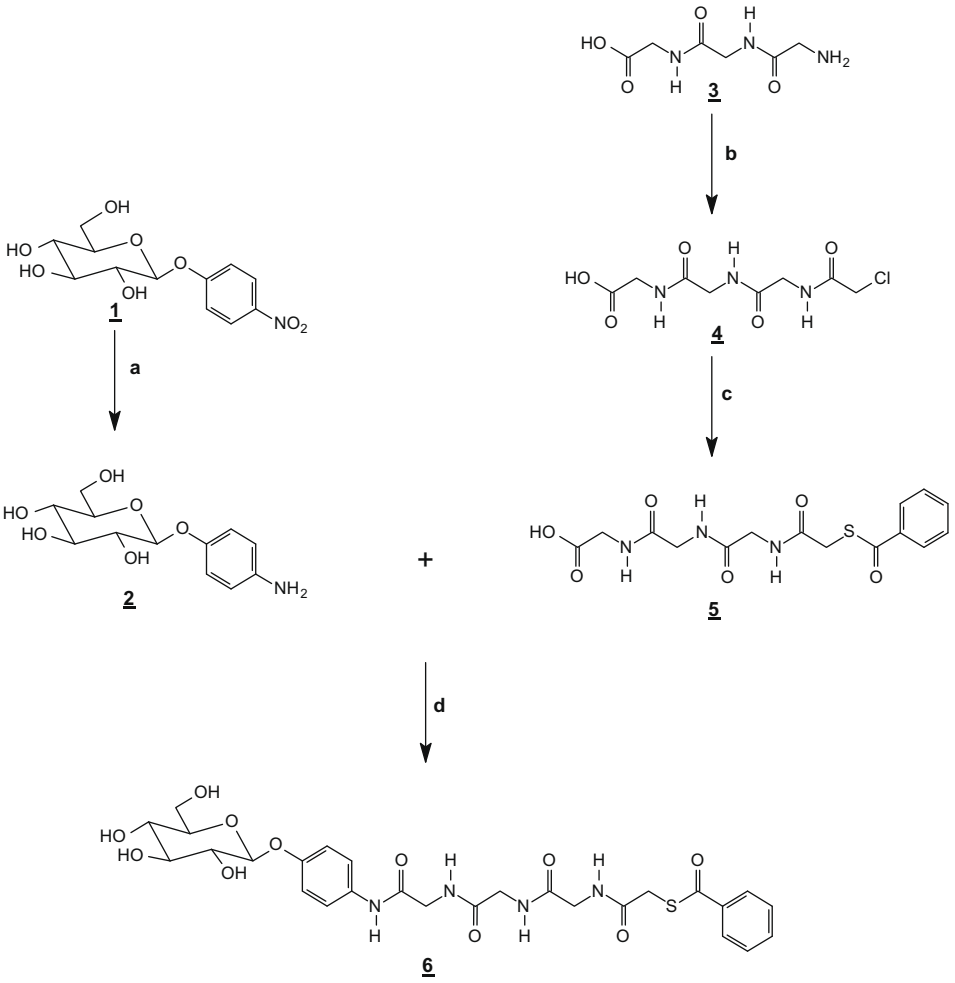

Scheme 1. Synthesis of Bz-MAG_3-G (**6**). Reagents and conditions: (a) H_2, Pd/C, methanol, yield: 100%; (b) chloroacetyl chloride, diethylether and water; (c) thiobenzoic acid, methanol, yield: 78%; two steps; (d) EDAC, dry DMF, yield: 32%.

The intermediate **2** was then reacted with benzoylated MAG_3 **5**, previously synthesized from **3**, using *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDAC) as coupling agent to obtain **6**. All compounds were characterized by ^1H NMR and ^{13}C NMR spectroscopy.[20] The technetium-99m labeled D-glucose-MAG_3 (^{99m}Tc-MAG_3-G) and the ^{99m}Tc-MAG_3 were prepared by ligand-exchange reaction with ^{99m}Tc-tartarate at pH 6–8. In this condition the benzoyl protecting groups of **5** and **6** are removed.[18]

After radiolabeling the products were purified by column chromatography on Florisil mesh 60–100, using, first, acetone to remove TcO_4^- and next, 0.9% saline to elute the ^{99m}Tc-MAG_3-G. The radiolabeling yields were determined by Instant Thin Layer Chromatograph (ITLC) on two systems: 0.9% saline to determine TcO_2 and acetone to determine TcO_4^-, as published elsewhere.[21] The radiochemical purities were higher than 90%.

Biodistribution of these complexes were performed in Ehrlich tumor-bearing Swiss mice (25–30 g) at 5, 30, 120 and 240 min post injection. The results are summarized in Tables 1 and 2. Both complexes were excreted rapidly through kidneys. The ^{99m}Tc-MAG_3-G had higher tumor-to-muscle (*T*/*M*) ratio than ^{99m}Tc-MAG_3 at all the times investigated (Table 3). It has been considered in the literature[22] that radiotracers that have a target/non-target ratio greater than 1.5 (50% greater capture in the target tissue) may be considered to be potential diagnostic agents. In a recent paper[1] a target/non-target ratio of about 3.0 was described for a derivative similar

Table 1
Biodistribution of ^{99m}Tc-MAG_3 in Ehrlich tumor-bearing mice (%ID/g)[a]

Tissue	5 min	30 min	120 min	240 min
Liver	7.62 ± 1.02	2.24 ± 0.19	1.58 ± 0.30	1.39 ± 0.15
Kidneys	20.43 ± 2.15	4.99 ± 1.35	2.64 ± 0.64	2.16 ± 0.44
Stomach	1.69 ± 0.39	2.08 ± 0.25	0.68 ± 0.12	1.43 ± 0.33
Blood	7.53 ± 0.45	1.05 ± 0.14	0.71 ± 0.17	0.74 ± 0.17
Bladder	19.74 ± 5.06	91.69 ± 5.64	51.96 ± 9.29	73.52 ± 6.55
Tumor	1.80 ± 0.42	0.68 ± 0.11	0.39 ± 0.09	0.38 ± 0.07
Muscle	1.47 ± 0.22	0.55 ± 0.05	0.34 ± 0.06	0.35 ± 0.07

[a] Injected dose 0.1 ml/370KBq. Data are expressed as percentage of %ID/g of tissue ± S.D. (n = 5).

Table 2
Biodistribution of ^{99m}Tc-MAG_3-G in Ehrlich tumor-bearing mice (%ID/g)[a]

Tissue	5 min	30 min	120 min	240 min
Liver	7.03 ± 1.07	3.69 ± 0.86	2.05 ± 0.46	2.23 ± 0.52
Kidneys	14.62 ± 4.50	3.72 ± 0.61	2.15 ± 0.40	2.54 ± 0.33
Stomach	3.46 ± 0.47	5.71 ± 0.20	4.24 ± 0.57	2.69 ± 0.51
Blood	6.09 ± 0.70	1.89 ± 0.37	0.72 ± 0.10	0.49 ± 0.07
Bladder	44.25 ± 8.87	93.95 ± 12.97	37.14 ± 8.10	55.83 ± 11.85
Tumor	2.25 ± 0.22	1.37 ± 0.11	0.50 ± 0.04	0.45 ± 0.04
Muscle	1.11 ± 0.13	0.63 ± 0.10	0.24 ± 0.03	0.21 ± 0.03

[a] Injected dose 0.1 ml/370KBq. Data are expressed as percentage of %ID/g of tissue ± S.D. (n = 5).

Table 3
Target/non-target ratio for ^{99m}Tc-MAG$_3$ and ^{99m}Tc-MAG$_3$-G[A]

Radiotracer	5 min	30 min	120 min	240 min
^{99m}Tc-MAG$_3$	1.22^a ± 0.14	1.22^a ± 0.10	1.16^a ± 0.08	1.10^a ± 0.11
^{99m}Tc-MAG$_3$-G	2.05^b ± 0.25	2.22^b ± 0.24	2.13^b ± 0.12	2.10^b ± .24

[A] The results are expressed as the mean ± S.D. (n = 5). The values were evaluated by the Tukey-Kramer test. Different letters indicate statistically significant differences.

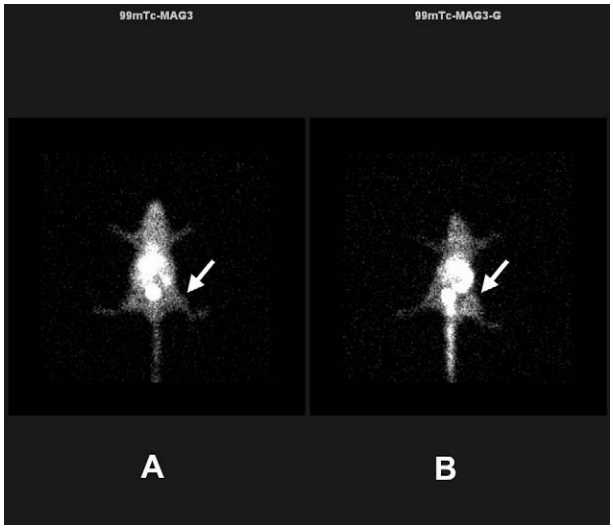

Figure 1. Scintigraphic images with Ehrlich tumor-bearing mice 240 min after injection of ^{99m}Tc-MAG$_3$ (A) and ^{99m}Tc-MAG$_3$-G (B). While under ketamine/xylazine anesthesia, 3.7 MBq of ^{99m}Tc-MAG$_3$ or ^{99m}Tc-MAG$_3$-G was injected into the tail vein.

to ^{99m}Tc-MAG$_3$-G in a tumor model. Similar results were also obtained by our research group using a glucose derivative without a phenolic group as spacer.[14] The data presented in this work were obtained with a molecule containing an aromatic group as spacer between the sugar and the ^{99m}Tc ligand, easily obtained from commercial available **1**.

By the scintigraphic images there were no differences between the right (tumor) and left (muscle) flanks in the uptake of ^{99m}Tc-MAG$_3$ (Fig. 1A). When the ^{99m}Tc-MAG$_3$-G complex was injected, a greater uptake of radioactivity by the right thigh occurred (Fig. 1B). Quantitative analysis of scintigraphic images obtained from Ehrlich tumor-bearing mice with ^{99m}Tc-MAG$_3$-G presented target/non-target ratios that were statistically higher than those of the ^{99m}Tc-MAG$_3$ complex (Table 4). These results showed the tropism of the ^{99m}Tc-MAG$_3$-G to the tumor during the whole experiment.

In summary, the D-glucose derivative **3** was coupled to Bz-MAG$_3$ **5**, and the product of this reaction (Bz-MAG$_3$-G, **6**) formed a complex with technetium-99m after removal of the benzoyl protecting group. After purification by column chromatography, a radiochemical purity superior to 90% was observed. The biodistribution studies and the scintigraphic images in animals showed that ^{99m}Tc-MAG$_3$-G had a higher affinity for the tumor than the ^{99m}Tc-MAG$_3$ complex. These data suggest that the ^{99m}Tc-MAG$_3$-G complex could be used as a possible agent for identification of tumors. Further studies will be carried out to evaluate the real potential of **6** for tumor diagnosis and will be reported in due course.

Table 4
Target/non-target ratio for ^{99m}Tc-MAG$_3$ and ^{99m}Tc-MAG$_3$-G obtained by gamma camera images (%ID/cm^2)[A]

Radiotracer	5 min	30 min	120 min	240 min
^{99m}Tc-MAG$_3$	1.23^a ± 0.02	1.09^a ± 0.07	1.18^a ± 0.12	1.13^a ± 0.13
^{99m}Tc-MAG$_3$-G	1.80^b ± 0.10	2.02^b ± 0.09	1.95^b ± 0.12	1.93^b ± 0.06

[A] The results are expressed as the mean ± S.D. (n = 3). The values were evaluated by the Tukey-Kramer test. Different letters indicate statistically significant differences.

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