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Synthesis and *In vitro* Evaluation of ^{99m}Tc-diglucosediethylenetriamine (DGTA) as a Potential Tumor Imaging Agent

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Using a single step chemical synthesis, we synthesized the potential tumor imaging agent ^{99m}Tc-diglucose-diethylenetriamine (DGTA) from diethylenetriamine and natural D-glucose. 10 min Incubation of 10 mg of precursor with 50 μg of $SnCl_2 \cdot 2H_2O$ at room temperature yielded over 95% of ^{99m}Tc labeling. The stability for 6 hours in saline or human plasma was over 90%. *In vitro* tumor cell uptake assays using the SNU-C5 and 9 L cell lines showed that, in 0-400 mg/dL glucose medium, cell uptake of ^{99m}Tc-DGTA was 1.5-8 times higher than that of [^{18}F]FDG. Moreover, [^{18}F]FDG uptake was dependent on glucose concentration in the medium, whereas cellular uptake of ^{99m}Tc-DGTA was not dependent on glucose concentration, suggesting that the two compounds have different uptake mechanisms by tumor cells.

Key Words : Glucose, ^{99m}Tc, SPECT, Tumor, Tumor imaging

Introduction

One of the standard imaging tools in tumor diagnosis is functional imaging by positron emission tomography (PET) with the radiolabelled glucose analog [^{18}F]fluorodeoxy-glucose (FDG). The metabolism of [^{18}F]FDG is similar to that of natural D-glucose. Tumors generally show high [^{18}F]FDG uptake because of increased glucose metabolism compared with normal cells due to the increased activity of hexokinase isoenzymes.[1,2]

Although [^{18}F]FDG is widely used for primary tumor detection, the production of [^{18}F]fluoride requires a cyclo-tron and the production of [^{18}F]FDG requires special facilities such as chemistry modules and hot-cells. More-over, [^{18}F]fluoride has a short half-life (110 min), limiting its use for long pharmacokinetic evaluations and late scan procedures.[3]

^{99m}Tc-labeled radiopharmaceuticals have a longer half-life (6 hours) and do not require high cost production facilities. ^{99m}Tc can be easily obtained from commercial ^{99}Mo/^{99m}Tc-generators and can be facilely prepared by reaction with a chelating group of ligands such as N_2S_2, N_4 and N_3S.[4,5] Although these chelating groups may alter the biological properties of their parent compounds, the low cost and easy preparation of ^{99m}Tc-labeled radiopharmaceuticals provide definite advantages.

To date, several ^{99m}Tc-labeled glucose derivatives have been prepared and tested, including ^{99m}Tc-ethylenedicysteine-deoxyglucose (ECDG),[6,7] ^{99m}Tc$(CO)_3$-deoxyglucose analogs,[5] ^{99m}Tc-1 and 5-thio-β-D-glucose,[8] ^{99m}Tc-DTPA-deoxyglucose[9] and ^{99m}Tc-S, MAG_3 and MAMA-deoxyglucose.[10,11] Although ^{99m}Tc-ECDG showed comparable uptake and glucose con-centration dependence as [^{18}F]FDG, the former had slow pharmacokinetics and high background in animal experi-ments. ^{99m}Tc-labeled thio glucose analogues also showed good tumor cell uptake, but it was lower than that of [^{18}F]FDG at physiological glucose concentrations and showed different biological characteristics than natural glucose. Other ^{99m}Tc-labeled glucose analogues have shown low tumor uptake in organs such as blood and lung.[11]

Radiolabeled di-, tri- and tetramer-RDG peptide analogues have shown high tumor uptake and favorable biological characteristics compared with monomer RGD peptide ana-logues in tumor cell and animal models.[12-14] Moreover, larger polymeric analogues had higher affinity for tumor sites than monomeric compounds from RDG analogues results, suggesting that radiolabeled di-glucose analogues may be used to target tumors. We therefore synthesized the di-glucose moiety ^{99m}Tc-diglucosediethylenetriamine from diethylenetriamine and natural D-glucose and evaluated its in vitro characteristics as a potential tumor imaging agent for single photon emission tomography (SPECT).

Results and Discussion

Synthesis of the Ligand and Formation of the ^{99m}Tc-complex. DGTA was synthesized as described (Figure 1), with a yield of 47.9%. It was chemically stable for 6 months at 0 °C.

The optimum conditions for labeling of ^{99m}Tc-DGTA consisted of 50 μg of $SnCl_2 \cdot 2H_2O$ and 10 mg of DGTA, with a labeling efficiency of 95.4 ± 2.7% at room temperature for 10 min. (n=3) To verify its chemical structure, we tried to

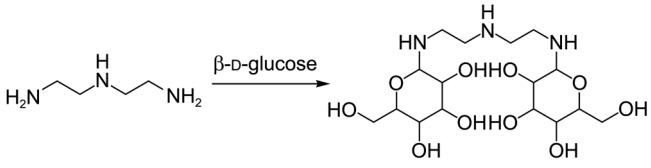

Figure 1. Scheme of DGTA synthesis.

Table 1. Cellular uptake (%); (n=3 per group)

	9 L (glioma)			SNU-C5 (colon carcinoma)		
Glucose concentration	$^{99m}TcO_4^-$	^{99m}Tc-DGTA	[^{18}F]FDG	$^{99m}TcO_4^-$	^{99m}Tc-DGTA	[^{18}F]FDG
0	1.25 ± 0.20	7.29 ± 0.76	5.31 ± 0.48	1.15 ± 0.05	7.72 ± 0.84	2.74 ± 0.15
100 (mg/dl)	1.04 ± 0.05	7.48 ± 1.43	1.55 ± 0.09	1.29 ± 0.13	8.01 ± 0.83	1.03 ± 0.02
450 (mg/dl)	1.13 ± 0.07	6.07 ± 0.21	1.18 ± 0.11	1.07 ± 0.07	6.33 ± 0.56	1.89 ± 0.45

synthesize Re-DGTA complex but we failed.

A kit containing a lyophilized mixture of 50 μg of $SnCl_2 \cdot 2H_2O$ and 10 mg of DGTA was chemically stable for 6 months at 0 °C, with a labeling efficiency of over 95%.

Although we could not synthesize Re-DGTA crystals for chemical structure analysis, we found that, as a ligand, DGTA showed strong binding to ^{99m}Tc and was easy to prepare.

***In vitro* Stability of ^{99m}Tc-DGTA.** The radiochemical purity of ^{99m}Tc-DGTA after incubation for 6 h in saline was 94.5 ± 2.7%, but was only 87.4 ± 3.7% after incubation in serum for 6 h. (n=3 for each group)

Tumor Cell Uptake Assay. *In vitro* tumor cell uptake of ^{99m}Tc-DGTA is shown in Table 1. In glucose free medium, the uptake of ^{99m}Tc-DGTA by these cell lines was higher than the uptake of [^{18}F]FDG. The ^{99m}Tc-DGTA showed the highest uptake on tumor cell compare with the previous ^{99m}Tc-labeled glucose analogues radiopharmaceuticals. In comparison, previously tested ^{99m}Tc-labeled glucose analogs showed tumor cell uptake similar to or lower than that of [^{18}F]FDG.

The previous compounds have one glucose moiety, whereas ^{99m}Tc-DGTA has two glucose moieties, suggesting that having multiple glucose moieties is associated with higher tumor cell uptake. From these results, larger polymeric analogues may have higher affinity for tumor sites than monomeric compounds like RGD peptide analogues.

Although [^{18}F]FDG uptake was markedly decreased at higher glucose concentrations, the uptake of ^{99m}Tc-DGTA was less dependent on glucose concentration. At very high glucose concentrations (450 mg/dl), uptake of ^{99m}Tc-DGTA was lower than low glucose concentration but remained higher than that of [^{18}F]FDG. Overall, uptake of ^{99m}Tc-DGTA was 1.5-8 times higher than that of [^{18}F]FDG, depending on the glucose concentration in the medium

When we attempted to inhibit tumor cell uptake of these radiotracers, we found that ^{99m}Tc-DGTA and [^{18}F]FDG showed different patterns. At 100 mg/dL glucose in the medium, the uptake of [^{18}F]FDG, *via* glucose transporter, was ~20% of its uptake under glucose free conditions. In contrast, ^{99m}Tc-DGTA showed similar or slightly higher tumor cell uptake at 100 mg/dl than in glucose-free medium, with decreased uptake observed only at 400 mg/dl glucose. These results indicate that these two compounds are taken up by different mechanisms.

Conclusion

We synthesized ^{99m}Tc-DGTA, a new potential tumor imaging agent, from β-D-glucose and diethylenetriamine, easily. This compound showed high labeling efficiency and stability in saline. Moreover, ^{99m}Tc-DGTA showed higher tumor cell uptake than [^{18}F]FDG, without being highly dependent on glucose concentration. These findings indicate that, although ^{99m}Tc-DGTA is a glucose analogue, its uptake mechanism differs from that of [^{18}F]FDG.

Experimental

Solvents and reagents were purchased from Sigma-Aldrich (Seoul, Korea). ^{1}H NMR spectra were obtained on a Varian Gemini 400 (Palo Alto, CA) and ^{13}C NMR spectra were recorded at 100 MHz. Chemical shifts are reported in parts per million (ppm, δ units) downfield from tetramethylsilane. Fast atom bombardment (FAB) mass spectra were obtained on a JMS-700 Mstation (Jeol Ltd., Tokyo, Japan). TLC and radioTLC were performed on Merck F254 silica plates and PALL ITLC SG, respectively and RadioTLC was analyzed using a Bioscan radio-TLC scanner (Washington, DC). [^{18}F]fluoride and [^{18}F]FDG were produced by the IBA Cyclone 18/9 cyclotron and FDG module (Belgium). $^{99m}TcO_4^-$ was obtained from Tyco ^{99}Mo/^{99m}Tc-generator (Tyco, The Netherlands).

Synthesis of Diglucosediethylenetriamine (DGTA) as a Ligand. DGTA was synthesized according to the previous paper with minor modification.[15] Briefly, diethylene triamine (1.57 mL, 14.4 mmol) was dissolved in methanol (20 mL) in an ice bath. To it was added 14.4 mL 1 M HCl/ether (14.4 mL) dropwise, the reaction mixture was stirred for 10 min and removed from the ice bath. Following the precipitation of a white solid, methanol (50 mL) was added and the reaction mixture was stirred for 20 min. To it was slowly added β-D-glucose (5.19 g, 28.8 mmol) dissolved in hot water (6 mL), and the mixture was incubated without stirring for 20 min. After stirring for 90 min, the resulting white solid precipitate was filtered and washed with methanol to removed impurities. We obtained 2.95 g of product in 47.9% yield. The synthesis scheme is shown in Figure 1. ^{1}H NMR (400 MHz, DMSO) σ 2.9-3.2 (10H, m), σ 3.2-3.4 (6H, m), σ 3.6 (2H, dd), σ 3.8 (2H, dd), σ 3.9 (2H, d) ^{13}C NMR (100 MHz, DMSO) σ 91.1, 78.4, 77.9, 74.1, 71.2, 62.0, 47.7, 42.3. MS (FAB) *m/z* 428 (m^+).

Radioisotope Labeling, Radiochemical Analysis and Kit Preparation. To determine the optimum amount of $SnCl_2 \cdot 2H_2O$ and the optimum amount of ligand, 370 MBq/0.3 mL $^{99m}TcO_4^-$ was added to a reaction vial containing 2-100 μL of $SnCl_2 \cdot 2H_2O$ (1 mg $SnCl_2 \cdot 2H_2O$/1 mL 1 N HCl)

and 1-10 mg of the ligand DGTA. After neutralization with 1 N NaOH, the mixture was incubated for 10 min at room temperature. Labeling efficiency was determined by radioTLC with ITLC, which were developed using acetone and saline, respectively. R_f value of ^{99m}Tc-DGTA is 0 in acetone and 1 with saline.

We also prepared lyophilized kits for easy preparation of ^{99m}Tc-DGTA. Each lyophilized kit contained 50 μg of $SnCl_2 \cdot 2H_2O$ and 10 mg of DGTA from radiolabeling experiments and also checked the kit stability to 6 months.

***In vitro* Stability of ^{99m}Tc-DGTA.** To 1 mL of human serum or saline was added 37 MBq/0.1 mL of ^{99m}Tc-DGTA, and the mixtures were incubated at 37 °C for 6 h. The stability of ^{99m}Tc-DGTA was analyzed at 3 and 6 h by radioTLC (n=3 each).

Tumor Cell Uptake Assay. Tumor cell uptake assay was performed to evaluate uptake of ^{99m}Tc-DGTA and to evaluate effect of glucose concentration on tumor cell uptake. The glioma cell line 9 L (ATCC; Manassas, VA, USA) and the colon cancer cell line SNU-C5 (Korean Cell Line Bank; Seoul, Korea) were cultured in RPMI 1640 (0.05% L-glutamine, 20 mM HEPES, 50 ug/mL penicillin-streptomycin, 10% fetal bovine serum). Cells (1×10^6/well) were placed in 6 well plates containing 0, 100, or 450 mg/dL D-glucose, to which were added 0.185 MBq ^{99m}Tc-DGTA, [^{18}F]FDG, and $^{99m}TcO_4^-$ (3 wells each). After incubation for 1 h, the cells were washed twice with phosphate-buffered saline and trypsinized. The radiopharmaceuticals contained cells were collected, and their radioactivity was counted using a gamma counter (COBRA II, Packard, U.S.A.).

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