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^{99m}Tc-glucoheptonate-guanine: Synthesis, biodistribution and imaging in animals

P. Unak,[1]* S. Teksoz,[1] F. Z. Biber Muftuler,[1] E. I. Medine,[1] C. Acar,[1] Y. Yurekli[2]

[1] *Department of Nuclear Applications, Institute of Nuclear Sciences, Ege University, 35100 Bornova Izmir, Turkey*
[2] *Department of Nuclear Medicine, School of Medicine, Adnan Menderes University, Aydin, Turkey*

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The aim of the current study was to design a nucleotide-based radiopharmaceutical which could be labeled with ^{99m}Tc and to investigate its radiopharmaceutical efficiency and stability. GHA (glucoheptonate) was used as bifunctional chelate. GHA was labeled with ^{99m}Tc by $SnCl_2$ reduction method first, and then G (guanine) was conjugated with ^{99m}Tc-GHA at 90 °C. In order to determine its radiopharmaceutical stability, thin layer radio chromatography (TLRC) and electrophoresis were employed. In addition, the results were confirmed using high performance liquid radio chromatography (HPLRC). Scintigraphic imaging was performed on rats with mammary tumors, while tissue distribution was determined on Albino Wistar rats. Labeling yield was found to be over 95% and the labeled complex maintained its stability during the study period. The lipophilicity of the ^{99m}Tc-GHG was measured and the partition coefficient (log*P*) of the labeled compound calculated. The results demonstrated that the uptake of ^{99m}Tc-GHG (^{99m}Tc-glucoheptonate-guanine) reached its maximum at 3 hours p.i. in stomach and intestines. Main way of excretion was renal. Hepatobiliary excretion was also observed. In conclusion, ^{99m}Tc-GHG may be useful as a nucleotide-based radiopharmaceutical for in vivo applications.

Introduction

Purines play a central role in cellular regulation and metabolism. They are substrates for DNA/RNA biosynthesis, regulators of the biosynthesis of some amino acids, and cofactors in the biosynthesis of phospholipids, sugar, and polysaccharides. Guanine is a purine base of a nucleotide found in DNA and RNA. It is known that DNA injury causes cell damage, plays an important and probably the central part in mutagenesis and carcinogenesis, and is a major contributor to ageing and age-related diseases.[1–5] O-substituted guanine derivatives are powerful agents used for tumor cell sensitization by inhibition of the DNA repair by the enzyme O-methylguanine-DNA methyltransferase (MGMT). To provide targeted accumulation of MGMT inhibitors in tumor tissue as well as tools for in vivo imaging, iodinated C8-alkyl-linked glucose conjugates of 2-amino-6-(5-iodothenyl)-9H-purine (O6-(5-iodothenyl) guanine, ITG) and 2-amino-6-(3-iodobenzyloxy)-9H-purine (O6-(5-iodobenzyl) guanine, IBG) were synthesized. It was reported that MGMT transferred alkyl groups from its O6-position, which was important in alkylating drug resistance.[6]

A group of chemicals which combine with DNA and interrupt its structure were used in cancer treatment. It has been indicated that ^{125}I labeled deoxyuridine, a thymine derivative, can be utilized to produce a high radiotoxic effect on the structure of DNA.[5,7] While many studies on radiolabeling of thymine have been published, there are hardly any references regarding the other DNA bases such as adenine, guanine and cytosine. Oligonucleotides are radiolabeled with beta and Auger electron emitting radionuclides such as ^{3}H, ^{35}S, ^{32}P, ^{125}I and ^{131}I for therapeutic purposes, whereas labeling with ^{123}I is useful for imaging.[3,4,8]

Over the past few years, radiolabeling of oligonucleotides has become a widespread research area in nuclear medicine. Due to the astonishing properties of DNA and RNA, labeled oligonucleotides have the potential to be used as radiopharmaceuticals. Thus, radiolabeled nucleotides can be used for purposes of imaging and management of tumors. Although the use of PET radionuclides is increasing, attention to ^{99m}Tc is still ongoing because of its decay characteristics, appropriate gamma-energy, short half-life, availability, low cost and variety of its chemistry. It was pointed out that ^{99m}Tc-L,L-ethylenedicysteine-guanine could be used as an imaging agent for tumor proliferation.[9]

^{99m}Tc-GHA is a radiopharmaceutical originally used for the assessment of renal function.[10] In addition to being a glucose analog as a substrate for energy,[11] it has been used for diagnosis of tumors such as those in brain and breast.[11,12]

The aim of the present study was to synthesize a guanine analog using GHA as a chelator and evaluate its radiopharmaceutical behavior and stability.

Experimental

Materials

$Na^{99m}TcO_4$ was obtained from a ^{99m}Tc/^{99}Mo generator (Monrol, Turkey). Guanine and glucoheptonic acid sodium salt were purchased from Sigma (Germany). All other chemicals were supplied from Merck Chemical Co. (Germany) and Aldrich Chemical

* E-mail: perihan.unak@ege.edu.tr

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Co. (Germany). Cellulose-coated plastic thin-layer chromatography (TLC) plates were purchased from Merck (Merck 5555, Germany). Bidistilled water was supplied by Millipore (Milli-Q Gradient A-10, USA) water purification instrument. Radioactivities were detected using Cd(Te) detector equipped with a RAD 501 (Turkey) single-channel analyzer.

Labeling procedure

Guanine was labeled with ^{99m}Tc using GHA as bifunctional chelating ligand.

^{99m}Tc-GHA: The GHA solution was prepared by dissolving 1 mg GHA in 1 ml H_2O and pH was adjusted to 6 using 1N NaOH solution. Then instantly prepared $SnCl_2$ solution (300 µl; 1 mg $SnCl_2$/1 ml H_2O) was added and mixed with 370 MBq (10 mCi) $^{99m}TcO_4^-$. The mixture was allowed to react for 20 minutes at ambient temperature to label GHA with ^{99m}Tc.

Inactive G stock solution: 5 mg G was dissolved in 2 ml of 0.1N NaOH and pH was adjusted to 6 using 1N HCl solution.

^{99m}Tc-GHG: The GHA solution was prepared by dissolving 1 mg GHA in 1 ml H_2O and pH was adjusted to 6 using 1N NaOH solution. Then 300 µl freshly prepared $SnCl_2$ solution (1 mg $SnCl_2$/1 ml H_2O) was added and mixed with 370 MBq (10 mCi) $^{99m}TcO_4^-$. One ml inactive G solution was added into labeled compound and the mixture was allowed to stand for five minutes and then heated at 90 °C for 15 minutes and cooled to room temperature. It was later filtered through the Millipore 0.22 µm membrane filter directly into a vacuum vial.

Quality control for ^{99m}Tc-G, ^{99m}Tc-GHA and ^{99m}Tc-GHG complexes was performed by TLRC, HPLRC and paper electrophoresis.

Chromatography

High performance liquid radio chromatography (HPLRC): The HPLC system consisted of a Shimadzu quaternary gradient pump (Model LC-10Atvp, Shimadzu, Japan), an automated syringe injector (20 µl loop) and a 5 µm reverse phase column (EXSAX-5-250AF Macherey-Nagel; Shimadzu, Japan). The column was eluted with 10% methanol, 10% acetonitrile, 80% water at 1 ml/min at 40 °C. The eluted components were detected at 210 nm by UV detector SPD-10A/V and by their gamma-radiation using Cd(Te) detector equipped with a RAD 501 single-channel analyzer.

Thin layer radio chromatography (TLRC): Physiological saline (0.9% NaCl), ACD (0.068M citrate, 0.074M dextrose, pH 5) and buffer solution (0.2M potassium phosphate, 2 mM mercaptoethanol, 0.4% TritonX100, pH 7) were used as developing media. When the solvent reached the desired distance along the strip in the developing medium, the strips were taken from the TLRC tank and dried at room temperature. Each TLRC sheet was covered with a celloband after its development and sliced into 0.5 cm widestrips. Then the radioactivity on the pieces was detected. TLRC chromatograms were obtained from these records by plotting counts versus distance. The R_f values and labeling yields were thus derived.

Electrophoresis: Electrophoresis was performed with a Gelman Electrophoresis Chamber supply using Whatman 3MM cellulose electrophoresis strips. Cathode and anode poles and application points were indicated on electrophoresis strips which were later moistened with saline solution (0.9% NaCl), ACD (0.068M citrate, 0.074M dextrose pH 5) and buffer solution (0.2M potassium phosphate, 2 mM mercaptoethanol, 0.4% TritonX100 pH 7). The application point was adjusted to the origin of the electrophoresis paper. The strips were placed in the electrophoresis chamber after the samples were applied to the strips. Standing time and applied voltage were 105 minutes and 300 volts, respectively. Developed strips were dried and cut into one cm pieces and each one was counted with the same detector. The same procedure was applied to $^{99m}TcO_4^-$, reduced ^{99m}Tc, ^{99m}Tc-GHA and ^{99m}Tc-GHG.

Lipophilicity (partition coefficient)

The lipophilicity (log*P*) of ^{99m}Tc-GHA and ^{99m}Tc-GHG was determined for the system n-octanol/water. 50 µl of each labeled compound was added into test tubes containing 3 ml of n-octanol in 3 ml of water. The tubes were shaken for an hour at room temperature and allowed to stand for phase separation. 0.5 ml aliquots of each phase were removed and counted. The partition coefficient was calculated as the ratio of cps/g of octanol to cps/g of water. Experiments were conducted in quadruplicate.

Stability in serum

In vitro stability of ^{99m}Tc-GHA and ^{99m}Tc-GHG in serum was determined by incubating 0.6 ml of each complex solution with 1 ml of human serum at 37 °C. The aliquots were then analyzed in time intervals of 1, 2, 3, 4 and 24 hours by TLRC and the radioactivity was counted.

Biodistribution studies on rats

Experiments on animals were approved by the Institutional Animal Review Committee of Ege University. ^{99m}Tc labeled product was sterilized by passing through a 0.22 µm membrane filter, then injected into the tail vein of male Albino Wistar rats 24 weeks of age and with a weight range of 150–200 g. The

injected mass of ^{99m}Tc-GHG was 1 μg/rat and injected activity was approximately 20 MBq/rat.

The rats were sacrificed under intense ether atmosphere after given intervals and their organs removed, weighted and counted by Cd(Te) detector.

Animal tumor model

Experiments on animals were approved by the Institutional Animal Review Committee of Ege University. Breast tumors were induced with DMBA in female Albino Wistar rats as described previously.[13–15] Tumor sizes of approximately 1 cm were measured. It has been reported that the tumors created by DMBA are considered to be ER+.[16]

Scintigraphic imaging studies on mammary tumor bearing Albino Wistar rats

The imaging studies were performed on healthy and mammary tumor bearing female Albino Wistar rats using Siemens e-Cam Single Camera (Siemens Medical Systems, USA) which was equipped with a low-energy high-resolution collimator.

The ^{99m}Tc labeled product was sterilized by passing through a 0.22 μm membrane filter and injected into the tail vein of female Albino Wistar rats with a weight range of 150–200 g. The injected mass of ^{99m}Tc-GHG was 1 μg/rat and injected activity was approximately 18.5 MBq/rat. A supplemental dose of alfazine and alfamine were used. Static images were obtained from anterior projection of 256×256 matrices for 5 minutes at 15, 20, 25, 30, 35, 40, 45, 50, 55, 60 and 90 minutes. For quantitative evaluation, regions of interests (ROIs in counts per pixel) were drawn on tumor site and muscle as background-activity and time-activity curves were generated. The ROI counts of tumor and muscle were used to calculate tumor/tissue ratios.

Statistical analysis

Differences in the mean values of measured activities were evaluated statistically by SPSS 10 program (Univariate Variance Analyses and Pearson Correlation). Probability values <0.05 were considered significant. Pearson correlation was carried out between tumor/muscle ratios of two tumors.

Results and discussion

Labeling yield was 95.7±1.1% ($n=10$). Quality control with TLRC, electrophoresis, HPLC and HPLRC showed that the complex was sufficiently pure. Chromatographic and electrophoresis experiments confirmed that there was only one product (Figs 1 and 2). Electrophoresis diagrams show that the labeled product has anionic structure as seen in Fig. 1.

Glucoheptonate was conjugated to the amino group of guanine under optimal conditions (pH 6). Although alternative structures such as GHA conjugated to NH_2 at position 2 of the guanine nucleus may occur, the reaction was conducted in an aqueous medium. It was reported that the amino function at position 2 has a stable resonance and the alkyl NH_2 is more reactive than that in position 2. Thus, we suggested that GHA was conjugated to the alkyl NH_2 group similar to the report by YANG et al.[9]

Theoretical N-octanol/water partition coefficient (log*P*) values are –5.68, –4.36 and –0.90 for GHG, GHA and G according to ACD/log*P* algorithm program, respectively.[17] Experimental N-octanol/water partition coefficients are –1.98±0.16 and –1.17±0.24 for ^{99m}Tc-GHG and ^{99m}Tc-GHA, respectively ($n=4$). ACD does not calculate the log*P* values for charged complexes because of shielding effect of attached groups.[17] ^{99m}Tc-GHA may display higher lipophilicity due to ^{99m}Tc conjugation to the hydroxyl groups of GHA. As a result significant discrepancy was found between theoretical lipophilicity of ligands and the experimental lipophilicity of their ^{99m}Tc complexes. Similar results have been reported previously in other studies.[15,18] As expected, the lipophilicity of G was reduced by GHA attachment. This means that GHG is less lipophilic than G and GHA. Therefore, lipophilicity of ^{99m}Tc-GHG is smaller than those of GHA and G.

^{99m}Tc-GHG maintained its stability in a neutral medium and at room temperature for five hours. The results of the serum stability experiments demonstrated that approximately 57% of ^{99m}Tc-GHG existed as an intact complex in human serum within 24 hours (Fig. 8).

According to the Table 1, the uptake of ^{99m}Tc-GHG reached maximum after 3 hours in stomach and intestines. Blood samples were collected from rats at 15, 45, 90 and 180 minutes p.i. of ^{99m}Tc-GHG and measured for radioactivity. The activity was cleared in an exponential manner (Fig. 3). Biological half-life was determined to be approximately 84 minutes. There was no significant uptake in lungs, heart, brain, spleen and pancreas. Lungs showed a biodistribution profile similar to that in blood. Therefore, the activity uptake in lungs was not specific. The activity ratio of liver to blood and muscle increased over time according to Fig. 4. Ratios increased to 5 for liver to blood and 12 for liver to muscle after 180 minutes. The activity ratio of kidney to blood and muscle increased with time, as shown in Figs 5 to 8 for kidney to blood and 22 for kidney to muscle. Afterwards, while blood activity decreased, kidneys and large intestines exhibited some activity since main excretion way was renal. However, hepatobiliar excretion was also seen. Furthermore, we measured the

thyroid uptake to determine the in vivo stability of the complexes, but could not observe remarkable radioactivity in the thyroid.

Gamma camera images after 15 minutes and 60 minutes are presented in Fig. 6. They show a significant amount of activity in the tumor site. Activity ratios of tumor to muscle were 3.9 for the upper tumor and 2.2 for the lower tumor within 1 hour, as seen in Fig. 7. Both tumors showed similar activity distribution profiles. Pearson correlation existed between tumor/muscle ratios of two tumors ($P = 99.99\%$).

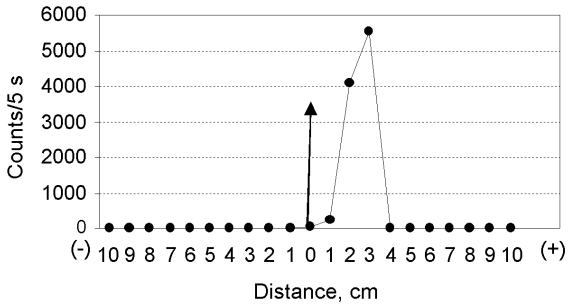

Fig. 1. Electrophoresis diagram of ^{99m}Tc-GHG

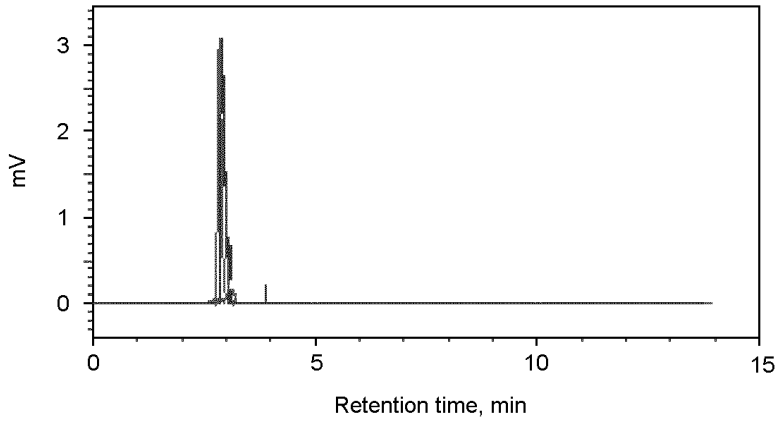

Fig. 2. HPLC and HPLRC chromatogram of ^{99m}Tc-GHG (the two lines belong to Cd(Te) radioactivity detector and UV detector, respectively)

Table 1. Biodistribution (in %dose/g) in rat tissues ($n = 3$)

Organ	15 minutes		45 minutes		90 minutes		180 minutes	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Heart	0.11	0.04	0.10	0.05	0.05	0.02	0.04	0.02
Lungs	0.36	0.17	0.27	0.16	0.16	0.06	0.10	0.06
Liver	0.28	0.18	0.23	0.14	0.29	0.09	0.50	0.29
Kidneys	0.28	0.16	0.33	0.18	0.40	0.19	0.89	0.49
S. Intestine	0.26	0.18	0.42	0.16	0.40	0.49	0.45	0.41
L. Intestine	0.62	0.54	0.43	0.32	0.86	0.47	9.14	3.13
Stomach	2.18	1.74	5.32	2.46	3.45	1.82	2.95	2.58
Spleen	0.09	0.06	0.10	0.04	0.05	0.02	0.07	0.03
Pancreas	0.25	0.15	0.16	0.04	0.13	0.08	0.09	0.03
Muscle	0.14	0.11	0.06	0.02	0.04	0.02	0.04	0.03
Testis	0.05	0.03	0.04	0.01	0.04	0.03	0.02	0.02
Prostate	0.35	0.17	0.27	0.07	0.13	0.03	0.17	0.06
Fat	0.16	0.17	0.06	0.04	0.14	0.01	0.10	0.06
Thyroid	0.29	0.26	0.33	0.23	0.29	0.14	0.35	0.00
Bladder	2.87	1.95	0.65	0.36	1.43	0.77	0.80	0.27
Brain	0.01	0.00	0.02	0.01	0.01	0.00	0.01	0.00
Blood	0.40	0.18	0.28	0.10	0.18	0.04	0.10	0.03

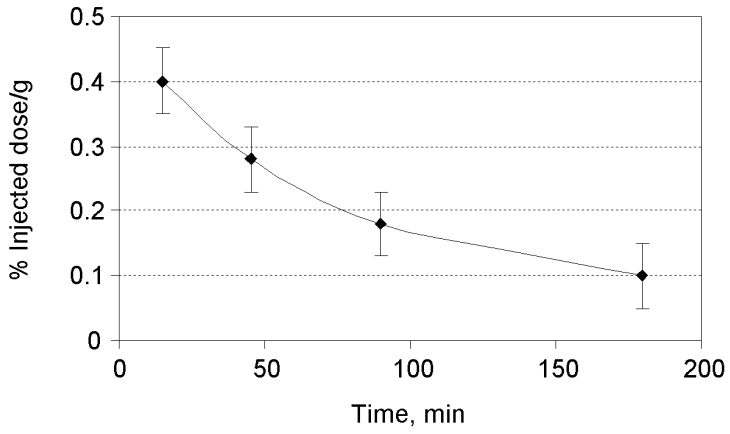

Fig. 3. Time-activity profile for ^{99m}Tc-GHG in blood

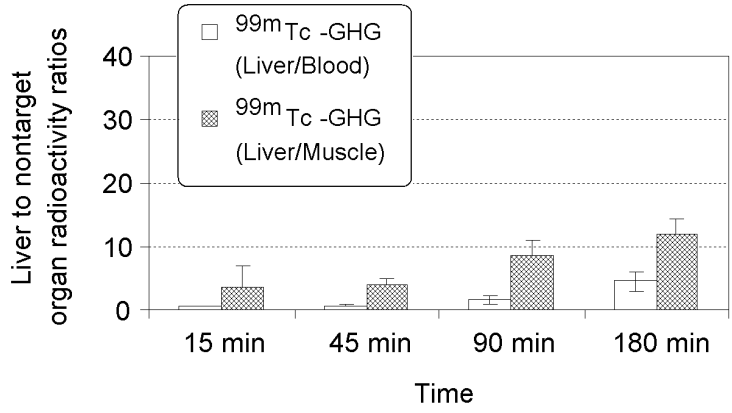

Fig. 4. Activity ratios of liver to blood and liver to muscle

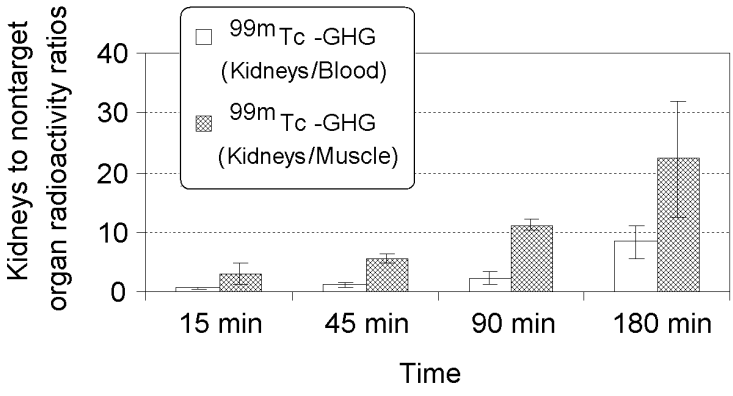

Fig. 5. Activity ratios of kidney to blood and kidney to muscle

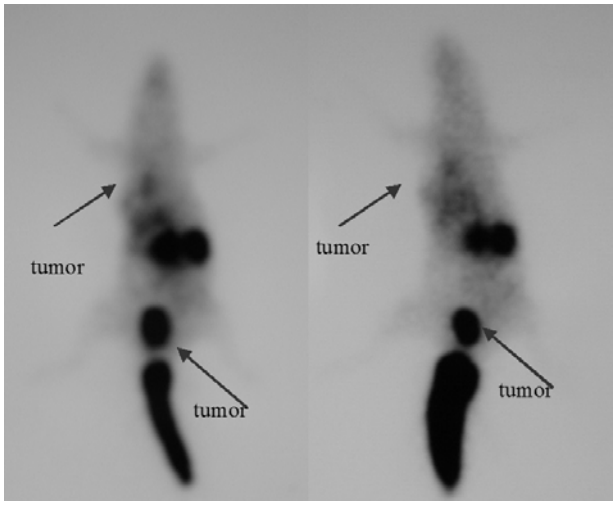

Fig. 6. Gamma camera image 15 minutes and 60 minutes postinjection (two tumors at right foreleg and left leg were induced on the imaged animal)

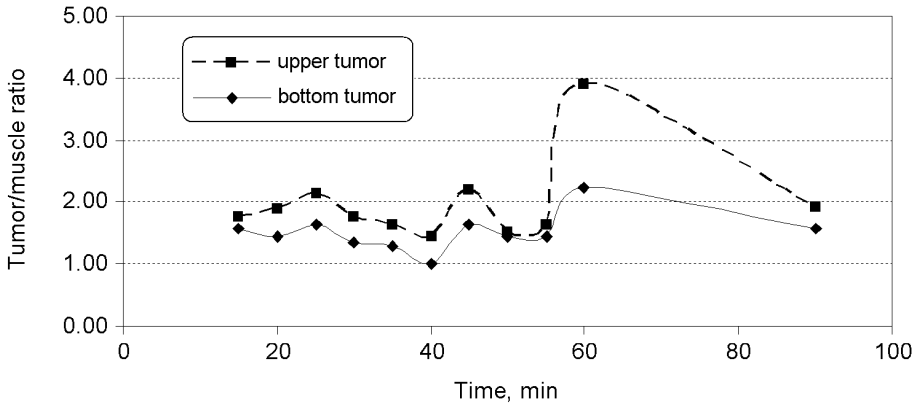

Fig. 7. Tumor/muscle ratios (two tumors at right foreleg and left leg were induced on the imaged animal)

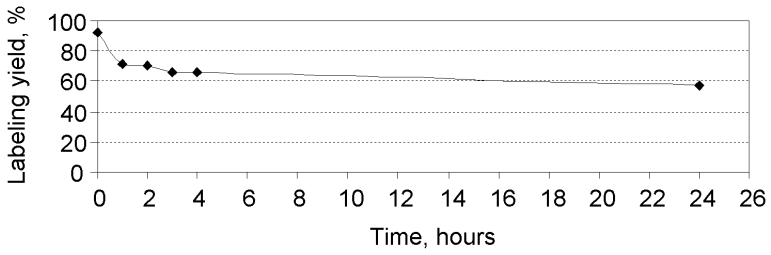

Fig. 8. Stability of ^{99m}Tc-GHG in serum

We have previously reported that a series of GHA peptide conjugates could be used for tumor targeting.[14,15,19] GHA is a small molecule and has been shown to be a stable chelator with ^{99m}Tc and ^{188}Re. Autoradiographic studies of ^{99m}Tc-GHA demonstrate rapid clearance of most of the injected radioactivity with the major excretory pathway through the kidney.[10] ^{99m}Tc-GHA has been proposed for static scintigraphy of the kidney and used to assess morphology, diagnose scars, cysts, and tumors, and has been used as an index of functioning renal parenchyma.[20] Here, we used ^{99m}Tc-GHA as bifunctional chelating agent to conjugate guanine to it.

Charge and lipophilicity affect the overall biodistribution, pharmacokinetics, metabolism and excretion characteristics as well as tissue uptake kinetics of the molecule, thus, change its biological behavior. Therefore, we expect that ^{99m}Tc-GHG may have different tumor and renal uptake. Negatively charged peptides apparently have a lower renal uptake in the environment of negatively charged membranes of tubular cells than neutral and cationic ones, consistent with the finding that positively charged amino acids can block peptide reabsorption by binding to the negatively charged membranes of tubular cells.[21] Concordantly, we observed that ^{99m}Tc-GHG had low kidney uptake as expected for a negatively charged complex. Low kidney uptake is very favorable during scintigraphy and radionuclide therapy since it will prevent renal radiotoxicity.

In the case of an ideal tumor-specific agent, the most desired feature is its distribution resulting in a target to non-target ratio greater than 3.0.[22] It was reported that tumor/muscle (T/M) ratios in ^{99m}Tc-EC-Guan (^{99m}Tc-ethylenedicysteine-guanine) and ^{99m}Tc-EC (^{99m}Tc-ethylenedicysteine) groups at 1–3.5 hours post injection were 3.1–4.0 and 2.5–2.7, respectively, in gamma-scintigraphic imaging studies in tumor-bearing rabbits.[9] We calculated the ratio as 2.2–3.9 within one hour in mammary tumor bearing rats, therefore, the labeled complex may have a potential for the imaging the breast or other tumors related to guanine metabolism.

Our data indicated that ^{99m}Tc-GHG is suitable for tumor imaging. Its simple chemistry also overcomes the complex chemistry of other nucleotide agents such as ^{18}[F]-FHBG (9-(4-^{18}[F] Fluoro-3-hydroxymethylbuthyl) guanine).[23] Although many other radiopharmaceuticals could be used for assessment of tumor proliferation and/or metabolic activity,[9,23,24] the choice should be made not only by the biological behavior of the radiopharmaceuticals, but also by its ease of preparation.

Conclusions

The labeling yield was satisfactory (appr. 96%) and ^{99m}Tc-GHG was stable in neutral medium and room temperature for 5 hours. Results demonstrated that ^{99m}Tc-GHG could be used for tumor targeting. Rapid uptake of ^{99m}Tc-GHG in the target tissue provides an advantage for imaging. The results of this study are sufficiently encouraging to bring about further evaluation of this or related ^{99m}Tc labeled nucleotide compounds for tumor imaging.

In summary, we have developed ^{99m}Tc-GHG, and our findings suggest that ^{99m}Tc-GHG may be useful as a nucleotide radiopharmaceutical for in vivo applications.

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References

1. M. K. DEWANJEE, M. KAPADVANJWALA, A. KRISHAN, A. N. SERAFINI, A. K. GHAFOURIPOUR, E. L. OATES, D. M. LOPEZ, G. N. SFAKIANAKIS, J. Clin. Immun., 16 (1993) 276.
2. M. K. DEWANJEE, Diagnostatic Oncology, 3 (1993) 189.
3. D. J. HNATOWICH Jr., P. WINNARD, F. VIRZI, M. FOGARASI, T. SANO, C. L. SMITH, C. R. CANTOR, M. RUSCKOWSKI, J. Nucl. Med., 36 (1995) 2306.
4. V. N. KARAMYCHEV, I. G. PANYUTIN, M. K. KIM, N. LE, C. H. PAIK, J. A. CARRASQUILLO, M. W. REED, R. D. NEUMANN, J. Nucl. Med., 41 (2000) 1093.
5. B. GUTFILEN, E. RODRIGUES, R. SORAGGI, L. H. B. DA FONSECA, Nucl. Med. Com., 22 (2001) 1133.
6. U. MUHLHAUSEN, R. SCHIRRMACHER, M. PIEL, B. LECHER, M. BRIEGERT, A. PIEE-STAFFA, B. KAINA, F. ROSCH, J. Med. Chem., 49 (2006) 263.
7. B. GUTFILEN, B. L. A. RIBEIRO, M. F. MATTOS, C. R. DASILVA, M. BERNARDO, Arquivos de Biologia e Tecnologia, 39 (1996) 69.
8. V. N. KARAMYCHEV, M. W. REED, R. D. NEUMANN, I. G. PANYUTIN, Acta Oncol., 39 (2000) 687.
9. D. J. YANG, K. OZAKI, C. S. OH, A. AZHDARINIA, T. YANG, M. ITO, A. GREENWE, J. BRYANT, S. KOHANIM, V. K. WONG, E. E. KIM, Pharm. Res., 22 (2005) 1472.
10. S. PERVEZ, A. MUSHTAQ, M. ARIF, Z. H. CHOHAN, J. Radioanal. Nucl. Chem., 256 (2003) 293.
11. Y. PEÑA, M. A. COCA, A. PERERA, J. F. BATISTA, M. L. BUSH, E. L. SA'NCHEZ, Clin. Nucl. Med., 30 (2005) 126.
12. S. BARAI, G. P. BANDOPADHAYAYA, P. K. JULKA, S. S. KALE, R. KUMAR, A. MALHOTRA, A. K. HALOI, A. SEITH, K. K. NAIK, H. DHANAPATHI, J. Clin. Neurosci., 12 (2005) 36.
13. F. Z. BIBER, P. UNAK, T. ERTAY, E. İ. MEDINE, F. ZIHNIOGLU, C. TASCI, H. DURAK, Appl. Radiation Isotopes, 64 (2006) 778.
14. T. ERTAY, P. UNAK, C. TASCI, F. Z. BIBER, F. ZIHNIOGLU, H. DURAK, J. Radioanal. Nucl. Chem., 265 (2005) 475.
15. T. ERTAY, P. UNAK, C. TASCI, F. Z. BIBER, F. ZIHNIOGLU, E. İ. MEDINE, H. DURAK, J. Radioanal. Nucl. Chem., 269 (2006) 21.
16. E. S. DELPASSAND, D. G. YANG, S. WALLACE, A. CHERIF, S. M. QUADRI, G. PRICE, A. GOUBERT, T. INOUE, D. A. PODOLOFF, J. Pharm. Sci., 85 (1996) 553.
17. R. MANNHOLD, H. WATERBEEMD, J. Computer-Aided Molecular Design, 15 (2001) 337.
18. T. ERTAY, P. UNAK, F. Z. BIBER, C. TASCI, F. ZIHNIOGLU, H. DURAK, Appl. Radiation Isotopes, 65 (2007) 170.
19. T. ERTAY, P. UNAK, C. TASCI, F. ZIHNIOGLU, H. DURAK, Appl. Radiation Isotopes, 62 (2005) 883.
20. H. MÄCKE, Schweizerische Medizinische Wochenschrift, 9 (1991) 299.
21. M. JONG, W. H. BAKKER, B. F. BERNARD, R. VALKEMA, D. J. J. KWEKKEBOOM, C. REUBI, A. SRINIVASAN, M. SCHMIDT, E. P. KRENNING, J. Nucl. Med., 40 (1999) 2081.
22. W. H. STRAUSS, A. NUNN, K. LINDER, J. Nucl. Cardiol., 2 (1995) 437.
23. D. E. PONDE, C. S. DENCE, D. P. SCHUSTER, M. J. WELCH, Nucl. Med. Biol., 31 (2004) 133.
24. D. A. MANKOFF, A. F. SHIELDS, J. M. LINK, M. M. GRAHAM, M. MUZI, L. M. PETERSON, J. Nucl. Med., 40 (1999) 614.