Evaluation of $^{99m}$Tc-CN5DG as a broad-spectrum SPECT probe for tumor imaging

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ARTICLE INFO

Keywords:
Glucose derivative
$^{99m}$Tc
Tumor probe
SPECT

ABSTRACT

Previously, we reported a $^{99m}$Tc(III)$^{+}$ labeled D-glucosamine derivative ($^{99m}$Tc-CN5DG) and evaluated it as a tumor imaging agent in mice bearing A549 tumor xenografts. In this paper, $^{99m}$Tc-CN5DG was further studied in U87 MG (human glioma cells), HCT-116 (human colon cancer cells), PANC-1 (human pancreatic cancer cells) and TE-1 (human esophageal cancer cells) tumor xenografts models to verify its potential application for imaging of different kinds of tumors. The biodistribution data showed that $^{99m}$Tc-CN5DG had a similar biodistribution pattern in four tumor models at 2h post-injection with high accumulation in tumors and kidneys. The tumor/muscle ratios (from 4.08 ± 0.42 to 9.63 ± 3.53) and tumor/blood ratios (from 17.18 ± 7.40 to 53.17 ± 16.16) of $^{99m}$Tc-CN5DG in four tumor models were high. All four kinds of tumors could be clearly seen on their corresponding SPECT/CT images. Pharmacokinetic study in healthy CD-1 mice demonstrated that $^{99m}$Tc-CN5DG cleared fast from blood (2 min, 12.97 ± 0.88%ID/g; 60 min, 0.33 ± 0.06%ID/g) and the blood distribution, elimination half-life was 5.81 min and 21.16 min, respectively. No abnormality was observed through the abnormal toxicity study. All of the above results demonstrated that $^{99m}$Tc-CN5DG could be a broad-spectrum SPECT probe for tumor imaging and its further clinical application is warranted.

Introduction

Nuclear medicine molecular imaging plays an increasingly important role in disease diagnosis and treatment over the past few decades [1, 2]. Positron Emission Tomography (PET) and Single Photon Emission Tomography (SPECT) are the two main imaging modalities that are used annually in the whole world. As we know, tumor cells need to consume more glucose to meet their enhanced metabolic rate. 2-[18F]fluoro-2-deoxy-D-glucose (18F)FDG is the most used PET radiopharmaceutical for various cancer diagnosis (such as lung cancer, breast cancer, lymphoma, esophageal cancer, pancreatic cancer) and staging due to its similar chemical structure to glucose [3-7]. However, in most developing countries, the use of [18F]FDG has limitation due to the need of a cyclotron for producing 18F isotope and high cost. In addition, another drawback of [18F]FDG is the fact that also non-FDG-avid tumors exist.

$^{99m}$Tc is the most used nuclide for SPECT because of its inexpensive cost and availability from the $^{99m}$Mo/$^{99m}$Tc generator. Besides, the overall number of SPECT scanners is far more than PET scanners in the whole world. Therefore, the development of $^{99m}$Tc labeled glucose derivatives as broad-spectrum SPECT probes for tumor imaging is necessary but remains challenging. Although $^{99m}$Tc labeled glucose derivatives for tumor imaging has been studied for about three decades, no one has been approved for routine use for tumor diagnosis in clinic [8-16].

Over the past ten years, we have been working on this field in order to develop a $^{99m}$Tc labeled glucose derivative with high tumor uptake and low background uptake [17-23]. Fortunately, a D-glucosamine derivative with an isonitrile group (CN5DG) was synthesized and radiolabeled with $^{99m}$Tc(III) to produce $^{99m}$Tc-CN5DG (Fig. 1). Preliminary studies in A549 tumor xenografts demonstrated that $^{99m}$Tc-CN5DG would be a powerful tool as a SPECT probe for tumor detection [24]. In order to verify the effectiveness of $^{99m}$Tc-CN5DG for detecting other kinds of tumor models, we conducted the evaluation of $^{99m}$Tc-CN5DG on four different human cell lines (U87 MG, human glioma cells; HCT-116, human colon cancer cells; PANC-1, human pancreatic cancer cells; TE-1, human esophageal cancer cells) to explore its feasibility as a broad-spectrum SPECT probe for tumor imaging. Moreover, its stability in vivo and pharmacokinetic properties are also studied.

Materials and methods

Materials and equipments

CN5DG kit which contains 0.5 mg CN5DG, 2.6 mg sodium citrate, 1 mg L-cysteine, 100 μg SnCl2•2H2O and 20 mg mannitol was obtained from Beijing Shihong Pharmaceutical Center, Beijing, China. Radiochemical purity was analyzed by a HPLC system equipped with a Waters...
2489 UV and a Gabi raytest radioactivity detector using an analytical column (18C, Kromasil, 100–5 μm, 250 × 4.6 mm). The HPLC gradient elution method was: (A, purified water; B, acetonitrile). 0–2 min, 10% B; 2–17 min, 10%–90% B; 17–20 min, 90% B; 20–25 min, 90%–10% B; 25–30 min, 10% B. 99mTcO4− was eluted from a 99Mo/99mTc generator (Atomic High Technology Co., Ltd, Beijing, China). Radioactivity was recorded by a Gamma Counter (WIZARD2400 2480 Perkin-Elmer system). SPECT/CT images were acquired on a small animal SPECT/CT scanner (Triumph SPECT/CT, console-gr157, USA).

Animals and tumor models

Balb/c nude mice (14–16 g) and CD-1 mice were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd (Beijing, China) and animal studies were conducted in compliance with the Regulations on Laboratory Animals of Beijing Municipality and the guidelines of the Ethics Committee of Beijing Normal University. HCT116, Panc-1 and TE-1 cells were purchased from Cell Bank of Chinese Academy of Sciences (Shanghai, China). U87 MG cells were obtained from Key Laboratory of Radiopharmaceuticals of Ministry of Education, Beijing Normal University. U87 MG, HCT116, Panc-1 and TE-1 cells were cultured in MEM, IMDM, DMEM and RPMI 1640 (ATCC modified) medium containing 10% PBS and 1% penicillin-streptomycin, respectively. Tumor xenografts bearing mice models were developed by injecting tumor cells (3–5 × 106 cells) into the right forelimb of balb/c nude mice subcutaneously. Biodistribution and SPECT/CT imaging studies were carried out when the tumors reached 0.3–0.6 cm in diameter after 7–14 days post-inoculation.

Biodistribution studies

99mTc-CN5DG was successfully prepared using a CN5DG kit according to previous published literature [24]. 99mTc-CN5DG (0.1 mL, 74 kBq) was injected into nude mice bearing different tumor xenografts (U87 MG, HCT116, Panc-1, TE-1, n = 3) via the tail vein. The animals were sacrificed at 2 h post-injection. The injected tissues or organs (such as heart, liver, tumor, lung, kidney) were removed, weighted and their radioactivity were recorded by a gamma counter. The uptake values were calculated as the percentage of the injected dose per gram (%ID/g). The uptake results were presented as average ± SD (standard derivation) of 3 animals.

SPECT/CT imaging studies

55.5–74 MBq of 99mTc-CN5DG was injected into mice (n = 3 for each tumor model) and the mice were scanned at 2 h post-injection using a small animal SPECT/CT scanner (3 D whole-body scan, MMP 919 collimator). SPECT/CT images were reconstructed by HisPECT software and analyzed by a Vivoquant 2.5 software.

Dynamic uptake SPECT studies of 99mTc-CN5DG was performed on nude mice bearing U87 MG tumor xenografts. 18.5 MBq of 99mTc-CN5DG was injected into the mice and the SPECT data were acquired continuously from 5 to 60 min after injection (half-body scan, MMP 930 collimator). The dynamic SPECT images were generated from every 5 min’s SPECT data. The Regions of Interest (ROIs) from tumor tissues and the ROIs from muscle were drawn on SPECT images.

Metabolic studies in vivo

99mTc-CN5DG (0.1–0.2 mL, 2 mCi) was injected into A549 tumor bearing nude mice (n = 3) via the tail vein. The mice were sacrificed at 30 min post-injection, then blood and tumor were collected. The blood was centrifuged at 10,000 rpm for 5 min. 300 μL of the supernatant was mixed with 600 μL of acetonitrile. The precipitated protein was removed by centrifugation and the supernatant was collected, concentrated and then analyzed by HPLC. For metabolic stability in tumor, the separated tumors were homogenized by a tissue-tearor (DREMEL, Model 3000) and the tumor homogenate was passed through a 0.22 μm membrane. To the filtrate, 600 μL of acetonitrile was added and the precipitated protein was removed by centrifugation, then the supernatant was collected, concentrated and analyzed by HPLC.

Pharmacokinetic characteristics

36 normal female CD-1 mice (18–22 g) were divided into 6 groups (6 mice for one group, 3 female mice and 3 male mice). 370 kBq of 99mTc-CN5DG was injected into the mice via the tail vein. The mice were killed at 2 min, 10 min, 30 min, 60 min, 120 min and 240 min post-injection.
The blood samples were collected, weighted and the radioactivity were recorded by a gamma counter. The blood uptake value was presented as%ID/g ± SD of 6 animals. The blood uptake-time curves were generated and analyzed by DAS 3.2.8 software.

Abnormal toxicity studies

For novel radiopharmaceuticals, the abnormal toxicity was within consideration. Thus, we carried out the toxicity of the solution of 99mTc-CN5DG according to the regulation of pharmacopoeia of People’s Republic of China (2015 Edition). The total volume of the reaction mixture was adjusted to 5mL. Then, 0.5mL of this solution were injected to healthy CD-1 mice (n = 5, 18–20 g) via the tail vein. The toxicity of the solution was determined by observing the death and survival of mice in the next 48 h.

Statistical analysis

The biodistribution data were calculated and analyzed with Microsoft Excel 2016 and Prism 5.01. All data were presented as average ± SD. To compare differences between two data, the one tail-paired student t-test was used. P < 0.05 indicated a statistically significant difference.

Results

Biodistribution studies

The biodistribution patterns (Fig. 2) were similar in all four kinds of tumor models with high accumulation in kidneys and tumors. Among the four tumor models, the uptake of 99mTc-CN5DG in HCT-116, PANC-1 and TE-1 tumors (from 0.95 ± 0.09%ID/g to 1.24 ± 0.28%ID/g) were higher than that in U87 MG tumors (0.42 ± 0.07%ID/g). The tumor/muscle ratios in U87 MG, HCT-116, PANC-1 and TE-1 were 4.02 ± 0.42, 4.53 ± 0.54, 9.63 ± 3.53 and 9.35 ± 3.38, respectively. The tumor/blood ratios in PANC-1 (41.41 ± 8.75) and TE-1 (53.17 ± 16.16) tumor models were significantly higher than that in U87 MG (17.18 ± 7.40) and HCT-116 (30.59 ± 9.62) tumor models. The high tumor/blood ratios in all four tumor models indicated that 99mTc-CN5DG has low blood uptake. Taken together, these results demonstrated that 99mTc-CN5DG has a significant uptake in a variety of tumors and would be more suitable for the detection of PANC-1 and TE-1 tumors.

SPECT/CT studies

SPECT/CT three-dimensional whole body images were acquired at 2h after the injection of 99mTc-CN5DG (74 MBq) via the tail vein (n = 3). From the SPECT/CT images (Fig. 3), tumors could be clearly seen in all four tumor models. The ROI ratios of the tumor sites versus corresponding non-tumor sites for U87 MG, HCT-116, PANC-1 and TE-1 were 3.09 ± 0.19, 4.73 ± 0.15, 5.51 ± 0.79 and 6.01 ± 1.37, respectively. Moreover, kidney and bladder could also clearly seen from the SPECT/CT images, suggesting its clearance way was through the urinary system. These results were in consistence with the biodistribution data and verified that 99mTc-CN5DG holds potential for the diagnosis of glioma, colon cancer, pancreatic cancer and esophageal cancer.

Dynamic SPECT images (5–60 min) in nude mice bearing U87 MG tumor xenografts were shown in Fig. 4. From the SPECT images (Fig. 4A), U87 MG tumors could be clearly seen from 5 min to 60 min post-injection. The tumor uptake value reached the highest before 30 min after injection and cleared slowly afterwards. The tumor/muscle ratios (ROI ratios) drawn from the SPECT images were shown in Fig. 4B. The values increased from 1.79 (5–10 min) to 2.89 (55–60 min) and reached a plateau at about 50 min post-injection.

Metabolic studies in vivo

To investigate the metabolic stability of 99mTc-CN5DG in blood and tumor in vivo, A549 tumor xenografts bearing nude mice were used. As shown in Fig. 5a, the retention time of co-injection (control and tumor) was 13.19 min, indicating that 99mTc-CN5DG kept intact in tumor. As shown in Fig. 5b, the retention time (13.57 min) of 99mTc-CN5DG in blood at 30 min after intravenous injection was consistent with the control (13.85 min), suggesting its stability in blood in vivo. Combined with our previous studies, we concluded that 99mTc-CN5DG was transported into tumor cells through glucose transporters and was not further metabolized in tumors [24].

Pharmacokinetic characteristics

The pharmacokinetic parameters were calculated by the statistical moment method of the two-compartment model using DAS 3.2.8 software. Time-activity curve of blood of 99mTc-CN5DG in healthy CD-1 mice was shown in Fig. 6. The blood uptake of 99mTc-CN5DG at 2min post-injection was 12.97 ± 0.88%ID/g while the uptake value was 0.33 ± 0.06%ID/g at 60 min post-injection. These data suggested that 99mTc-CN5DG had a fast clearance rate in blood in vivo. Major pharmacokinetics parameters are listed in Table 1. The blood distribution half-life was 5.81 min and the blood elimination half-life was 21.16 min.

Abnormal toxicity studies

In the abnormal toxicity study, none of five mice showed abnormality or died after 48 h. If one takes a person weighing 60 kg, the dose received by a mouse (20 g) was about 300 times as much as a human received per kilogram, suggesting the low toxicity of the solution of 99mTc-CN5DG.
Discussion

Developing a $^{99m}$Tc labeled glucose derivative for tumor detection has been of great importance. In our previous studies, $^{99m}$Tc-CN5DG was evaluated in nude mice bearing A549 tumor and would be a potential agent for tumor imaging. In this study, we explored its application in U87 MG, HCT-116, PANC-1 and TE-1 tumor models and its pharmacokinetic characterization in vivo. $^{99m}$Tc-CN5DG could be readily prepared by using a CN5DG kit (Fig. 1). When the pH value of the kit was adjusted to about 6.0, the RCP of the product was more than 95%. Lower pH value (pH < 4.0) is not suitable because isonitrile is not stable under highly acidic conditions. Sodium citrate is a necessary compound in the CN5DG kit to stabi-
lize Sn2⁺ and ⁹⁹mTcO₄⁻·nH₂O would be formed without it. Commonly, 740 MBq-1110 MBq of ⁹⁹mTc labeled radiopharmaceuticals would be used to perform a SPECT scan in clinic, so we studied the radioactivity amount of ⁹⁹mTcO₄⁻ to prepare ⁹⁹mTc-CNSDG. As a result, when the radioactivity of ⁹⁹mTcO₄⁻ was up to 3700 MBq, the RCP of ⁹⁹mTc-CNSDG was still over 95%.

In our previous biodistribution studies in A549 tumor bearing mice, ⁹⁹mTc-CNSDG had the highest uptake in tumors at 30 min post-injection and the tumor/muscle ratio was high at 60 min post-injection, but the uptakes in blood and other tissues (such as heart, stomach) were also high at 60 min. It should be noted that the tumor/muscle and tumor/blood ratios were both high at 2 h post-injection. Based on these data, in this study, we performed the biodistribution and SPECT imaging studies at 2 h post-injection to get ideal tumor/non-target ratios. The biodistribution data in four tumor models revealed that ⁹⁹mTc-CNSDG had a moderate tumor uptake (from 0.42 ± 0.07%ID/g to 1.24 ± 0.28%ID/g) and high tumor/muscle (from 4.08 ± 0.42 to 9.63 ± 3.53), tumor/blood ratios (from 17.18 ± 7.40 to 53.17 ± 16.16). These data were in accordance with that in A549 tumor model (tumor uptake, 1.48±0.23%ID/g; tumor/muscle ratio, 29.68 ± 3.12; tumor/blood ratio, 60.79 ± 2.86) [24]. The uptake of ⁹⁹mTc-CNSDG in U87 MG tumor was significantly lower than that in other three kinds of tumors, possibly because the amounts of Gluts that transport the tracer into U87 MG tumor cells are lower than those on the other three tumor cell lines. This needs further investigation.

The SPECT/CT images of ⁹⁹mTc-CNSDG verified its broad-spectrum application in U87 MG, HCT-116, PANC-1 and TE-1 tumor models. The ROIs (regions of interest drawn from SPECT images) ratios of the tumor sites versus corresponding non-tumor sites for U87 MG, HCT-116, PANC-1 and TE-1 were 3.09 ± 0.19, 4.73 ± 0.15, 5.51 ± 0.79 and 6.01 ± 1.37, respectively. These data nearly consisted with the tumor/muscle ratios (from 4.08 ± 0.42 to 9.63 ± 3.53) from biodistribution data. Moreover, there is no observable background uptake on SPECT images, suggesting the low uptake in blood, which is also consistence with the biodistribution data (tumor/blood ratios varied from 17.18 ± 7.40 to 53.17 ± 16.16). In addition, the kidney/tumor ratios in TE-1 and PANC-1 tumor bearing mice are 1.97 and 1.67 from the biodistribution data, while the ROIs ratios of kidney/tumor from SPECT images in TE-1 and PANC-1 are 2.01 and 1.64. The biodistribution data also corresponded with the data drawn on SPECT images. Although U87 MG tumor uptake (0.42 ± 0.07%ID/g) was lower than the uptake in HCT-116, PANC-1 and TE-1 tumors (from 0.95 ± 0.09%ID/g to 1.24 ± 0.28%ID/g), the U87 MG tumor could also be clearly seen in the whole-body SPECT/CT images. Dynamic SPECT images further verified that the U87 MG tumors could be detected at 5 min after the injection of ⁹⁹mTc-CNSDG. These results warranted the early tumor detection ability of ⁹⁹mTc-CNSDG in other three kinds of tumor models (HCT-116, PANC-1 and TE-1). When compared to [¹⁸F]FDG, ⁹⁹mTc-CNSDG has very low physiological uptake in the brain (0.02%ID/g). As presented in Fig. 1, ⁹⁹mTc-CNSDG is a positively charged complex, while [¹⁸F]FDG is a neutral molecule. Hence, [¹⁸F]FDG can cross the brain-blood barrier easily while ⁹⁹mTc-CNSDG can’t. So little uptake of ⁹⁹mTc-CNSDG was observed in mouse brain. As a result, the tumor/blood ratio of ⁹⁹mTc-CNSDG in U87 MG tumor bearing mice was high (19.00 ± 0.71). This findings may compensate the deficiency of [¹⁸F]FDG for brain tumor detection.

The evaluation of ⁹⁹mTc-CNSDG as a tumor imaging agent was initially studied in A549 tumor bearing nude mice (including tumor uptake studies, biodistribution studies, SPECT/CT imaging studies) [24], as a continuous study of this work, A549 tumor bearing mice were used for the metabolic study of ⁹⁹mTc-CNSDG in vivo. In this study, ⁹⁹mTc-CNSDG was also found to be stable in blood and had no other metabolite in A549 tumors, suggesting it exhibited high stability in vivo. The significant tumor uptake, fast blood clearance and good safety of ⁹⁹mTc-CNSDG warranted its further clinical application for cancer diagnosis.

**Conclusion**

In this study, ⁹⁹mTc-CNSDG exhibited moderate tumor uptake, tumor/muscle and tumor/blood ratios in U87 MG, HCT-116, PANC-1 and TE-1 tumor models. SPECT/CT images in four tumor models demonstrated that all four kinds of tumors could be clearly detected. Moreover, ⁹⁹mTc-CNSDG was stable in blood and tumors in vivo and rapidly cleared from blood. These findings verified ⁹⁹mTc-CNSDG could be used as a promising broad-spectrum tumor imaging agent and had potential for clinical translation.

**Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**CRediT authorship contribution statement**

**Xuran Zhang**: Conceptualization, Methodology, Validation, Investigation, Writing - original draft. **Qing Ruan**: Investigation, Validation. **Yuhao Jiang**: Investigation, Validation. **Qianqian Gan**: Investigation, Validation. **Junbo Zhang**: Conceptualization, Validation, Resources, Writing - review & editing, Supervision.

**Funding**

This work is financially supported, in part, by the National Natural Science Foundation of China (21771023, 22076013) and the project of the Beijing Municipal Science and Technology Commission (Z181100002218033) and China Postdoctoral Science Foundation (212400211).
References

[1] P. Brugarolas, J. Comstock, D.W. Dick, T. Ellmer, J.W. Engle, S.E. Lapi, S.H. Liang, E.E. Parent, P.N.V. Kishore, S. Selivanova, et al., Fifty years of radiopharmaceuticals, J. Nucl. Med. Technol. 48 (Suppl 1) (2010) 345–395.

[2] J.K. Willmann, N. van Bruggen, L.M. Dinkelborg, S.S. Gambhir, Molecular imaging in drug development, Nat. Rev. Drug. Discov. 7 (7) (2008) 591–607.

[3] A. Niyonkoru, X. Chen, K.H. Bakari, D.N. Wimalaratne, A. Boushari, M.M.R. Amour, X. Lan, Evaluation of the diagnostic efficacy of 18F-fluorine-2-deoxy-o-glucose PET/CT for lung cancer and pulmonary tuberculosis in a tuberculosis-endemic country, Cancer Med. 9 (3) (2020) 931–942.

[4] E. Acar, B. Turpaz, S. Yigit, G. Kaya, Comparison of the volumetric and radioisotopes findings of 18F-FDG PET/CT images with immunohistochemical prognostic factors in local/locally advanced breast cancer, J. Nucl. Med. Commun. 40 (7) (2019) 764–772.

[5] T. Ni, P. Perrot, C. Fraj, C. Delannoy, F. Liberman, P. Mir, B. Da Silva, M. Fragomeni, P.P. Ieria, G. Scambia, A. Giordano, et al., Evaluation of dual-time-point 18F-FDG PET/CT imaging for lymph node staging in vulvar cancer, J. Nucl. Med. 58 (12) (2017) 1913–1919.

[6] K. Sasaki, Y. Uchikado, H. Okumura, I. Omoto, Y. Kita, T. Arigami, Y. Unosono, T. Osaki, K. Maemura, S. Natsugoe, Role of 18F-FDG PET/CT in esophageal squamous cell carcinoma after neoadjuvant chemoradiotherapy, Anticancer Res. 37 (2) (2017) 859–864.

[7] J.W. Lee, C.M. Kang, H.J. Choi, W.J. Lee, S.Y. Song, J.-H. Lee, J.D. Lee, Prognostic value of metabolic tumor volume and total lesion glycolysis on preoperative 18F-FDG PET/CT in patients with pancreatic cancer, J. Nucl. Med. 55 (6) (2014) 898–904.

[8] X. Chen, L. Li, F. Liu, B. Liu, Synthesis and biological evaluation of technetium-99m-labeled deoxyglucose derivatives as imaging agents for tumor, Bioorg. Med. Chem. Lett. 18 (21) (2008) 5503–5506.

[9] J. Liang, Y. Chen, Z. Huang, Y. Zhao, L. He, Early chemotherapy response evaluation in tumors by 99mTc-DTPA-DG, Cancer Biother. Radiopharm. 23 (3) (2008) 363–370.

[10] A.L. Branco de Barros, V.N. Cardoso, LdG Mota, E.A. Leite, M.C. de Oliveira, R.J. Alves, Synthesis and biological evaluation of technetium-labeled glucose-MAG3 derivative as agent for tumor diagnosis, Bioorg. Med. Chem. Lett. 19 (9) (2009) 2497–2499.

[11] R. Iapuzeta, R. Castelli, M. Fernandez, J.A. Chabalgoity, M. Moreno, J.P. Gambini, P. Cabral, W. Forcal, Biological evaluation of glucose and deoxyglucose derivatives radiolabeled with 99mTc(CO3)(H2O)7+ core as potential melanoma imaging agents, Bioorg. Med. Chem. Lett. 21 (13) (2011) 7102–7106.

[12] S.J. Oh, J.-S. Ryu, E.-J. Yoon, M.S. Bae, S.J. Choi, K.B. Park, D.H. Moon, 99mTc-labeled 1-thio-β-o-glucose as a new tumor-seeking agent: synthesis and tumor cell uptake assay, Appl. Radiat. Isot. 64 (2) (2006) 207–215.

[13] J. Ding, H. Su, F. Wang, T. Chu, A pre-targeting strategy for imaging glucose metabolism using technetium-99m labeled dibenzocyclooctyne derivative, Bioorg. Med. Chem. Lett. 29 (14) (2019) 1791–1798.

[14] S. Singh, S. Singh, R.K. Sharma, A. Kaul, R. Mathur, S. Tomar, R. Varshney, A.K. Mishra, Synthesis and preliminary evaluation of a 99mTc labeled deoxyglucose complex (99mTc(DTPA-bis(DG)) as a potential SPECT based probe for tumor imaging, New J. Chem. 44 (7) (2020) 3062–3071.

[15] D.J. Yang, C.-G. Kim, N.R. Schechter, A. Azadharinia, D.-F. Yu, C.-S. Oh, J.L. Bryant, J.-J. Won, E.E. Kim, D.A. Podoloff, Imaging with 99mTc ECIDG targeted at the multifunctional glucose transport system: feasibility study with rodents, Radiology 226 (2) (2003) 465–473.

[16] N.B. Schechter, W.D. Erwin, D.J. Yang, E.E. Kim, R.F. Munden, K. Forster, L.C. Taing, J.D. Cox, H.A. Macapinlac, D.A. Podoloff, Radiation dosimetry and biodistribution of 99mTc-ethylene dicysteine-deoxyglucose in patients with non-small cell lung cancer, Eur. J. Nucl. Med. Mol. Imaging 36 (10) (2009) 1583–1591.

[17] T. Liu, J. Zhang, X. Wang, J. Yang, Z. Tang, J. Lu, Radiolabeled glucose derivatives for tumor imaging using SPECT and PET, Curr. Med. Chem. 21 (1) (2014) 24–34.

[18] T. Liu, Q. Gan, J. Zhang, Z. Jin, W. Zhang, Y.Zhang, Synthesis and biodistribution of novel 99mTc complexes of glucose dihalochromate as potential probes for tumor imaging, MedChemComm 7 (7) (2016) 1381–1386.

[19] T. Liu, Q. Gan, J. Zhang, Macro cyclic triamine derived glucose analogues for 99mTc(CO), labeling: synthesis and biological evaluation as potential tumor imaging agents, Chem. Biol. Drug Des. 89 (2) (2017) 277–284.

[20] X. Lin, Z. Jin, J. Ren, Y. Fang, W. Zhang, J. Hua, X. Wang, J. Zhang, Y. Zhang, Synthesis and biodistribution of a new 99mTc-oxy complex with deoxyglucose dichioro-3-bis(sodium 5-methyl-3,7,12-tetraoxo-8-oxa-8-azabicyclo [4.4.0] deca-4,7-dien-1-carboxylic acid) as a potential tumor imaging agent, Bioorg. Med. Chem. Lett. 19 (10) (2009) 2752–2754.

[21] X. Zhang, N. Gan, Q. Ruan, X. Xiao, J. Zhang, Evaluation and comparison of 99mTc-labeled D-glucosamine derivatives with different 99mTc cores as potential tumor imaging agents, Appl. Organomet. Chem. (2020), doi:10.1002/occ.6008.

[22] X. Zhang, Q. Ruan, X. Qian, G. Song, X. Fang, X. Lin, J. Du, J. Zhang, Novel 99mTc-labeled glucose derivative for single photon emission computed tomography: a promising tumor imaging agent, Mol. Pharm. 15 (8) (2018) 3417–3424.