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ORIGINAL ARTICLE

Evaluation of ^{64}Cu-labeled DOTA-D-Phe^{1}-Tyr^{3}-octreotide (^{64}Cu-DOTA-TOC) for imaging somatostatin receptor-expressing tumors

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Abstract
Objective In-111 (^{111}In)-labeled octreotide has been clinically used for imaging somatostatin receptor-positive tumors, and radiolabeled octreotide analogs for positron emission tomography (PET) have been developed. Cu-64 (^{64}Cu; half-life, 12.7 h) is an attractive radionuclide for PET imaging and is produced with high specific activity using a small biomedical cyclotron. The aim of this study is to produce and fundamentally examine a ^{64}Cu-labeled octreotide analog, ^{64}Cu-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid-D-Phe^{1}-Tyr^{3}-octreotide (^{64}Cu-DOTA-TOC).
Methods ^{64}Cu produced using a biomedical cyclotron was reacted with DOTA-TOC for 30 min at 45°C. The stability of ^{64}Cu-DOTA-TOC was evaluated in vitro (incubated with serum) and in vivo (blood collected after administration) by HPLC analysis. Biodistribution studies were performed in normal mice by administration of mixed solution of ^{64}Cu-DOTA-TOC and ^{111}In-DOTA-TOC and somatostatin receptor-positive U87MG tumor-bearing mice by administration of ^{64}Cu-DOTA-TOC or ^{64}Cu-1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid-octreotide (^{64}Cu-TETA-OC). The tumor was imaged using ^{64}Cu-DOTA-TOC, ^{64}Cu-TETA-OC, and FDG with an animal PET scanner.
Results ^{64}Cu-DOTA-TOC can be produced in amounts sufficient for clinical study with high radiochemical yield. ^{64}Cu-DOTA-TOC was stable in vitro, but time-dependent transchelation to protein was observed after injection into mice. In biodistribution studies, the radioactivity of ^{64}Cu was higher than that of ^{111}In in all organs except kidney. In tumor-bearing mice, ^{64}Cu-DOTA-TOC showed a high accumulation in the tumor, and the tumor-to-blood ratio reached as high as 8.81 ± 1.17 at 6 h after administration. ^{64}Cu-DOTA-TOC showed significantly higher accumulation in the tumor than ^{64}Cu-TETA-OC. ^{64}Cu-DOTA-TOC PET showed a very clear image of the tumor, which was comparable to that of ^{18}F-FDG PET and very similar to that of ^{64}Cu-TETA-OC.
Conclusions ^{64}Cu-DOTA-TOC clearly imaged a somatostatin receptor-positive tumor and seemed to be a potential PET tracer in the clinical phase.

Keywords ^{64}Cu · DOTA-D-Phe^{1}-Tyr^{3}-octreotide (DOTA-TOC) · Somatostatin receptor

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Introduction

Somatostatin receptors are expressed on neuroendocrine tumors, including carcinoid tumor, pituitary adenoma,

pheochromocytoma, and medullary thyroid carcinoma. Somatostatin receptors are also positive on the cell surfaces of other types of tumors, such as small cell lung carcinoma, meningioma, astrocytoma, and neuroblastoma. Recently, radiolabeled somatostatin analogs have been clinically and widely used, and In-111 (^{111}In)-labeled diethylenetriaminepentaacetic acid-octreotide (^{111}In-DTPA-OC) has been approved and is routinely used for the localization and staging of neuroendocrine tumors [1, 2]. Octreotide analogs have also been labeled with positron emitters because of the advantage of positron emission tomography (PET) [3–6].

Cu-64 (^{64}Cu; half-life, 12.7 h) is an attractive radionuclide for PET imaging, which decays by electron capture (41%), β^- (0.573 MeV, 40%) and β^+ (0.656 MeV, 19%). ^{64}Cu can be produced with high specific activity using a small biomedical cyclotron installed in hospitals or PET centers [7, 8]. Furthermore, ^{64}Cu is also potentially applicable to therapy, either by itself or replaced by another copper radionuclide, copper-67 (^{67}Cu; half-life, 61.7 h), which emits β^- rays (0.395–0.577 MeV) and γ rays (0.091–0.185 MeV). Therefore, ^{64}Cu-labeled octreotide analogs are promising tracers for PET imaging in patients with somatostatin receptor-positive tumors. ^{64}Cu-TETA-OC or its analogs (where TETA is 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid) showed high tumor accumulation in tumor-bearing mice or rats [9, 10]. In a study of humans, more lesions were reported to be seen with ^{64}Cu-TETA-OC PET than with ^{111}In-DTPA-OC SPECT [4].

1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) is one of the most useful chelators and has been widely used for the labeling of many radiometals, including ^{64}Cu. However, it has been reported that ^{64}Cu-DOTA complexes are not so stable, since ^{64}Cu tends to dissociate from the chelator followed by binding to copper-binding proteins [11, 12]. Thus, TETA has been extensively used as a stable chelator [4, 13], and more stable chelators have been developed [14, 15]. However, these new chelators are still in the research phase and are difficult to use routinely in the clinical phase. On the other hand, yttrium-90 (^{90}Y)-labeled DOTA-D-Phe^1-Tyr^3-octreotide (DOTA-TOC) has been evaluated in phase 1 and phase 2 clinical trials for therapy, meaning that radiolabeled DOTA-TOC would be easy and safe to apply to the clinical phase. In this study, ^{64}Cu-DOTA-TOC was prepared and its stability, biodistribution, and metabolism were evaluated and compared with those of ^{111}In-DOTA-TOC. The tumor accumulation of ^{64}Cu-DOTA-TOC in somatostatin receptor-positive tumor-bearing mice was compared with that of ^{64}Cu-TETA-OC. Finally, ^{64}Cu-DOTA-TOC PET imaging of tumor-bearing mice was performed. Based on the results, the usefulness and clinical applicability of ^{64}Cu-DOTA-TOC were discussed.

Materials and methods

General

$^{111}InCl_3$ (74 MBq/mL in 0.02 N HCl) was purchased from Nihon Medi-Physics (Nishinomiya, Japan). ^{64}Cu was produced with a biomedical cyclotron CYPRIS HM-18 (Sumitomo Heavy Industries Ltd., Tokyo, Japan) at our university hospital. ^{18}F was also produced at our hospital using the same cyclotron, and then ^{18}F-FDG was synthesized using an automated apparatus. DOTA-TOC was purchased from Bachem (Bubendorf, Switzerland). For the preparation of TETA-OC, octreotide was constructed using Fmoc-based solid-phase synthesis and TETA was conjugated to it as previously described [10]. Human glioblastoma cell line U87MG, expressing somatostatin receptor [16], was purchased from American Type Culture Collection (ATCC, Manassas, VA). Reversed-phase HPLC (RP-HPLC) analyses were performed with a C18 column (Capcell Pak C18 MG-II, 4.6 × 150 mm, Shiseido Co. Ltd., Tokyo, Japan) eluted with a linear gradient of a 20–30% mixture of acetonitrile and 0.1% aqueous TFA. Size-exclusion HPLC (SE-HPLC) analyses were performed with a TSKgel Super SW3000 column (4.6 × 300 mm, Tosoh, Tokyo, Japan) eluted with 0.1 M phosphate buffer (pH 6.8). TLC analyses were performed with silica plates (Silica gel 60, Merck, Darmstadt, Germany) with 10% aqueous ammonium acetate–methanol (1:1) as the developing solvent. Other reagents were of reagent grade and used as received.

Radiolabeling

For the preparation of ^{111}In-DOTA-TOC, 40 μL of $^{111}InCl_3$ (1.5 MBq) was incubated in 60 μL of 0.25 M acetate buffer (pH 5.5) for 5 min at room temperature, then DOTA-TOC (20 μg/20 μL of 0.25 M acetate buffer) was added and incubated for 30 min at 45°C. ^{64}Cu (200–300 MBq) was provided in a dry state and was dissolved in 100 μL of 0.25 M acetate buffer. Then DOTA-TOC (50 μg/150 μL of 0.25 M acetate buffer) or TETA-OC (50 μg/150 μL of 0.25 M acetate buffer) was added and incubated for 30 min at 45°C. The radiochemical purities of ^{64}Cu-DOTA-TOC, ^{64}Cu-TETA-OC, and ^{111}In-DOTA-TOC were determined by RP-HPLC and TLC. Rf values of ^{64}Cu-DOTA-TOC and ^{64}Cu were 0.5 and 0, respectively, by TLC analysis.

In vitro and in vivo stabilities

For the evaluation of in vitro stability, ^{64}Cu-DOTA-TOC (100 ng/20 μL of 0.1 M phosphate buffer) was added to 180 μL of murine serum, and the solution was incubated at

37°C for 6 h. The radioactivity of the sample was analyzed by SE-HPLC and RP-HPLC. For the evaluation of in vivo stability, blood was drawn from the hearts of mice at 5 min, 1 h, and 6 h after the administration of ^{64}Cu-DOTA-TOC (200 ng/100 μL). After centrifugation at 3,000 rpm for 10 min at 4°C, the resultant serum samples were filtered through a polycarbonate membrane with a pore diameter of 0.45 μm. The radioactivity of the sample was analyzed by SE-HPLC. RP-HPLC analysis was performed after filtering through a 10-kDa cutoff ultrafiltration membrane (VIVA-SPIN 500; Sartorius, Goettingen, Germany). Bovine serum albumin (BSA) was used as a molecular weight marker of SE-HPLC and eluted at 17 min.

Biodistribution study

The animals were cared for and treated in accordance with the guidelines of the animal care and experimentation committee of our university. Tumor-bearing mice were prepared by implanting U87MG tumor cells (5×10^6 cells) into the flanks of BALB/c nude mice. When tumors were palpable, the mice were used for biodistribution studies. A mixed solution of ^{64}Cu-DOTA-TOC (10 kBq) and ^{111}In-DOTA-TOC (30 kBq) (volume: 100 μL, total peptide dose: 200 ng) was administered to normal ddY mice, and ^{64}Cu-DOTA-TOC (10 kBq) or ^{64}Cu-TETA-OC (10 kBq) (volume: 100 μL, total peptide dose: 100 ng) was administered to U87MG tumor-bearing nude mice. At selected time points after administration, animals were killed and tissues of interest were excised and weighed. Their radioactivity was then measured with a well-type gamma counter (ARC-7001; Aloka Co. Ltd., Tokyo, Japan). Briefly, the total radioactivity of ^{64}Cu and ^{111}In was measured. The radioactivity of ^{111}In was measured 6 days after first measurement, since the count of ^{64}Cu was negligible at that time. The radioactivity of ^{64}Cu was calculated using these two measurements.

Urine and feces samples were collected using metabolic cages (Metabolica TYPE MM-ST; Sugiyama-Gen Iriki Co. Ltd., Tokyo, Japan) at 6 and 48 h after administration of ^{64}Cu-DOTA-TOC alone or mixed with ^{111}In-DOTA-TOC. Urine samples were also drawn from the bladder at 30 min

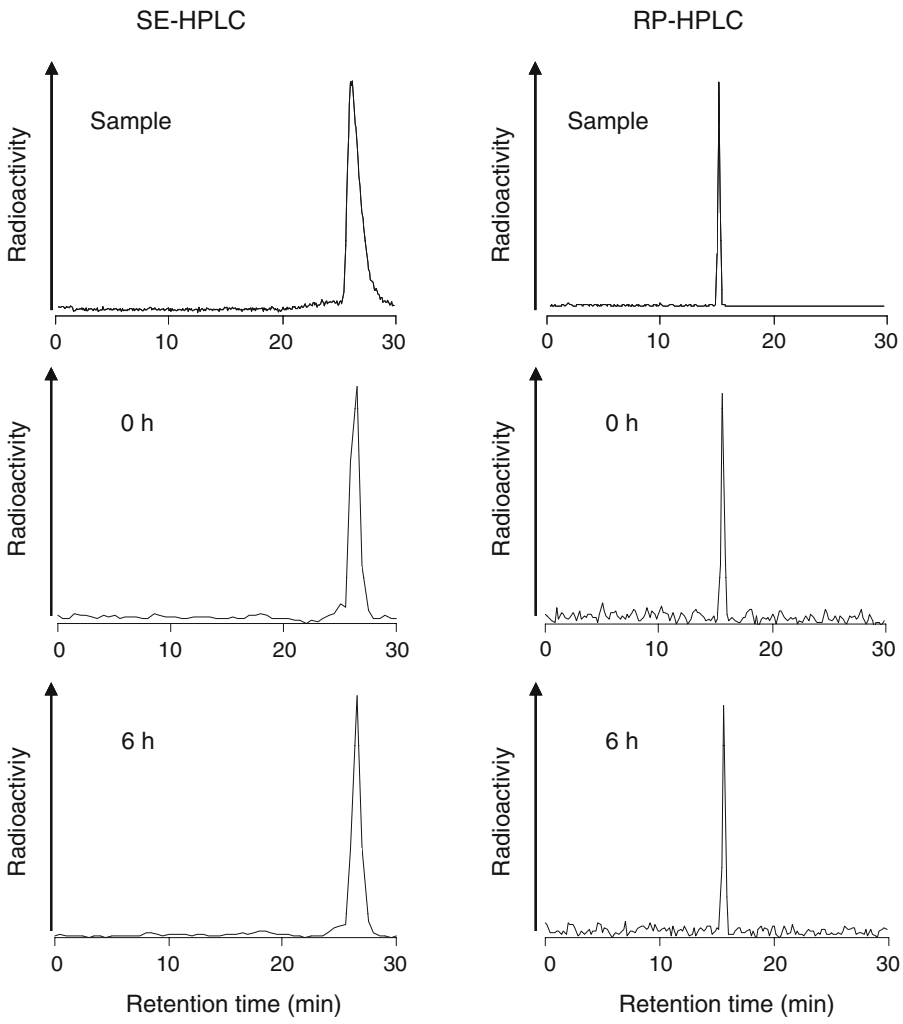

Fig. 1 Radioactivity profiles of ^{64}Cu-DOTA-TOC after incubation in murine serum. After incubation in murine serum at 37°C for 0 and 6 h, the radioactivity of the sample was analyzed by SE-HPLC and RP-HPLC. Retention time of ^{64}Cu-DOTA-TOC was 26 min by SE-HPLC and 16 min by RP-HPLC

and 6 h after administration. The radioactivity of urine was analyzed by SE-HPLC.

PET imaging

PET imaging was performed using an animal PET scanner (Inveon; Siemens AG, Munich, Germany). After fasting for about 12 h, U87MG tumor-bearing mice were injected intravenously with ^{18}F-FDG (20 MBq) and imaged at 1 h after administration. Two days after ^{18}F-FDG PET, mice were injected intravenously with ^{64}Cu-DOTA-TOC (20 MBq) or ^{64}Cu-TETA-OC (20 MBq) and imaged at 6 and 24 h after administration.

Statistical analysis

Data are expressed as means ± standard deviations where appropriate. Results were analyzed using the unpaired *t* test. Differences were considered statistically significant when *p* values were less than 0.05.

Results

Radiolabeling

The radiolabeling yield of ^{64}Cu-DOTA-TOC was more than 95% for all five times radiolabeling. The radiolabeling yields of ^{64}Cu-TETA-OC and ^{111}In-DOTA-TOC were also more than 95%.

In vitro and in vivo stabilities

All radioactivities were recovered after filtration through a polycarbonate membrane. After the incubation in murine serum at 37°C for 6 h, ^{64}Cu-DOTA-TOC existed only as the intact form, and the retention times of SE-HPLC and RP-HPLC were 26 and 16 min, respectively (Fig. 1). In contrast, time-dependent transchelation to protein (retention time 18–19 min) was observed after administration to mice (60.6 ± 3.8 and 95.2 ± 1.4% at 1 and 6 h, respectively) (Fig. 2).

Biodistribution study

^{64}Cu-DOTA-TOC showed rapid blood clearance and renal accumulation, similar to that of ^{111}In-DOTA-TOC in normal mice at an early time point after administration (Table 1). However, ^{64}Cu-DOTA-TOC showed retention in the blood after 1 h, and consequently the radioactivity of ^{64}Cu in all organs except kidney was much higher than that of ^{111}In. ^{64}Cu-DOTA-TOC showed significantly high accumulation in the liver and intestine compared with

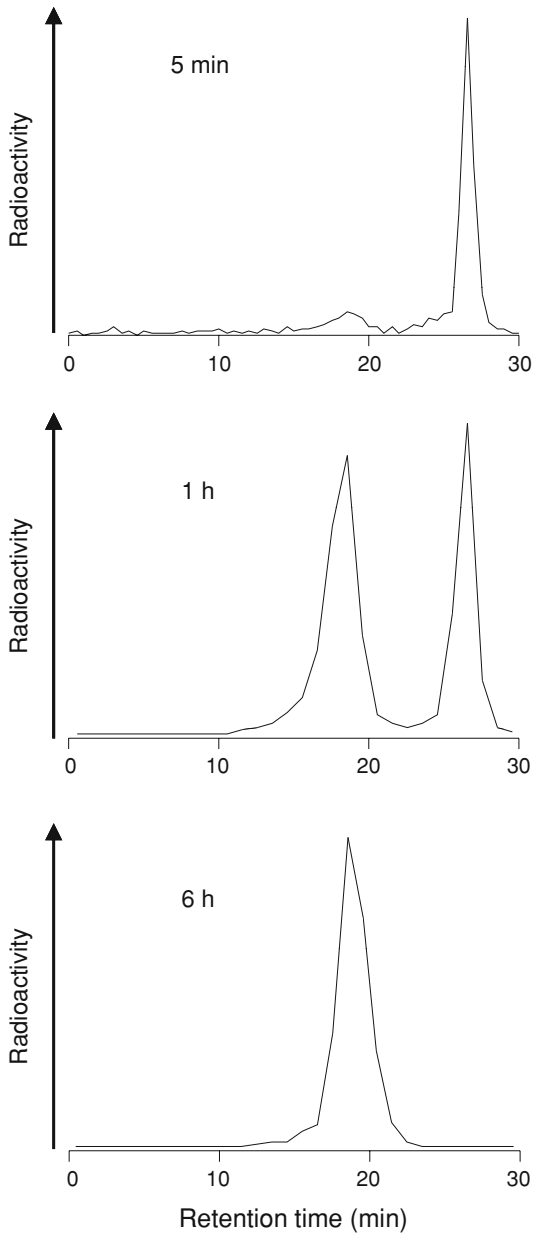

Fig. 2 Radioactivity profiles in the blood after administration of ^{64}Cu-DOTA-TOC to mice. Blood was drawn from the hearts of mice at 5 min, 1 h, and 6 h after the administration of ^{64}Cu-DOTA-TOC and the radioactivity was analyzed by SE-HPLC

^{111}In-DOTA-TOC ($p < 0.001$ at all time points). ^{64}Cu-DOTA-TOC showed steady clearance from the kidney.

^{64}Cu-DOTA-TOC showed high accumulation and retention in the tumors of U87MG tumor-bearing mice, resulting in a tumor-to-blood ratio of 8.81 ± 1.17 at 6 h after administration (Table 2). The tumor-to-muscle ratios were as high as 38.9 ± 13.8 and 45.1 ± 12.5 at 3 and 6 h, respectively.

By 48 h after administration with ^{64}Cu-DOTA-TOC, 67.0 ± 5.3 and 12.8 ± 9.3% of radioactivity were excreted

Table 1 Biodistribution of ^{64}Cu-DOTA-TOC and ^{111}In-DOTA-TOC in normal mice

	Time after injection				
	10 min	30 min	1 h	3 h	6 h
^{64}Cu-DOTA-TOC					
Blood	3.37 ± 0.13	1.35 ± 0.31	0.60 ± 0.03**	0.48 ± 0.09**	0.46 ± 0.02**
Liver	2.15 ± 0.20**	2.68 ± 0.73**	2.53 ± 0.63**	3.26 ± 0.51**	2.61 ± 0.36**
Kidney	16.53 ± 2.77	11.76 ± 2.70	7.60 ± 0.76	4.18 ± 0.74**	3.88 ± 0.53**
Intestine	1.06 ± 0.02**	1.16 ± 0.17**	1.40 ± 0.27**	2.40 ± 0.21**	1.71 ± 0.18**
Spleen	0.84 ± 0.37	0.51 ± 0.27	0.33 ± 0.20	0.34 ± 0.14	0.26 ± 0.12
Pancreas	2.48 ± 0.13**	1.79 ± 0.07**	1.75 ± 0.13**	1.11 ± 0.16**	0.49 ± 0.16
Lung	3.49 ± 0.25	2.40 ± 0.41	1.92 ± 0.58*	2.67 ± 0.25**	2.36 ± 0.33**
Heart	1.59 ± 0.14	0.81 ± 0.42	0.47 ± 0.24	0.61 ± 0.21*	0.49 ± 0.18
^{111}In-DOTA-TOC					
Blood	3.30 ± 0.22	1.27 ± 0.43	0.34 ± 0.04	0.08 ± 0.06	0.04 ± 0.00
Liver	0.92 ± 0.04	0.48 ± 0.11	0.26 ± 0.03	0.24 ± 0.05	0.16 ± 0.02
Kidney	15.41 ± 3.14	11.78 ± 3.03	9.72 ± 1.59	11.91 ± 1.50	9.51 ± 1.11
Intestine	0.79 ± 0.04	0.42 ± 0.08	0.27 ± 0.08	0.38 ± 0.25	0.35 ± 0.20
Spleen	1.21 ± 0.11	0.50 ± 0.09	0.23 ± 0.05	0.13 ± 0.03	0.13 ± 0.01
Pancreas	1.50 ± 0.07	1.01 ± 0.12	0.61 ± 0.07	0.37 ± 0.04	0.27 ± 0.01
Lung	3.46 ± 0.23	1.46 ± 0.41	0.52 ± 0.08	0.21 ± 0.03	0.20 ± 0.05
Heart	1.38 ± 0.03	0.60 ± 0.22	0.17 ± 0.06	0.08 ± 0.03	0.06 ± 0.02

Each value represents the mean % injected dose/g of organ ± SD of 4 animals

Significant difference from ^{111}In-DOTA-TOC (* $p < 0.01$, ** $p < 0.001$)

Table 2 Biodistribution of ^{64}Cu-DOTA-TOC in U87MG tumor-bearing mice

	Time after injection			
	30 min	1 h	3 h	6 h
Blood	1.53 ± 0.09	0.80 ± 0.01	0.59 ± 0.14	0.52 ± 0.14
Liver	3.69 ± 0.53	4.27 ± 1.23	3.51 ± 0.83	3.43 ± 0.69
Kidney	16.95 ± 2.02	15.12 ± 0.97	9.83 ± 1.18	6.52 ± 0.89
Intestine	1.27 ± 0.03	1.65 ± 0.20	2.17 ± 0.34	2.21 ± 0.52
Muscle	0.34 ± 0.04	0.20 ± 0.05	0.12 ± 0.03	0.11 ± 0.04
Tumor	2.51 ± 0.13	3.43 ± 1.03	4.43 ± 0.43	4.50 ± 0.76
Tumor-to-blood ratio[a]	1.64 ± 0.07	4.31 ± 1.31	7.70 ± 1.30	8.81 ± 1.17
Tumor-to-muscle ratio[a]	7.5 ± 1.2	17.7 ± 3.3	38.9 ± 13.8	45.1 ± 12.5

Each value represents the mean ± SD of three animals. Mean tumor weight was 23 mg

[a] Expressed as % injected dose/g of organ

in urine and feces, respectively. There was no significant difference between ^{64}Cu-DOTA-TOC and ^{111}In-DOTA-TOC in the radioactivity of urine at 30 min or 6 h after injection (Fig. 3a). Radioactivity was not observed in the protein fraction in the urine at 30 min or 6 h after administration of ^{64}Cu-DOTA-TOC, and about 60–70% of radioactivity was the intact peptide (Fig. 3b), similar to the case with ^{111}In-DOTA-TOC (data not shown).

PET imaging

The tumors were clearly visible with both ^{64}Cu-DOTA-TOC and ^{18}F-FDG (Fig. 4). ^{64}Cu-DOTA-TOC showed heterogeneous accumulation in the tumor, and accumulation of radioactivity was observed in the same region at 6–24 h. Relatively uniform uptake of ^{18}F-FDG was seen throughout the tumor. ^{64}Cu-DOTA-TOC showed high accumulation in the liver and bladder at 6 h, and the radioactivity was retained in the liver and was cleared from the bladder at 24 h after administration.

Comparison studies with ^{64}Cu-TETA-OC

^{64}Cu-DOTA-TOC showed significantly higher accumulation than ^{64}Cu-TETA-OC in the tumor, and also in the blood, liver, kidney, and intestine (Fig. 5a). The tumor-to-organ ratios were almost the same in all organs except for muscle (Fig. 5b). The tumors were clearly visible with both ^{64}Cu-DOTA-TOC and ^{64}Cu-TETA-OC (Fig. 5c), and the whole body images were very similar.

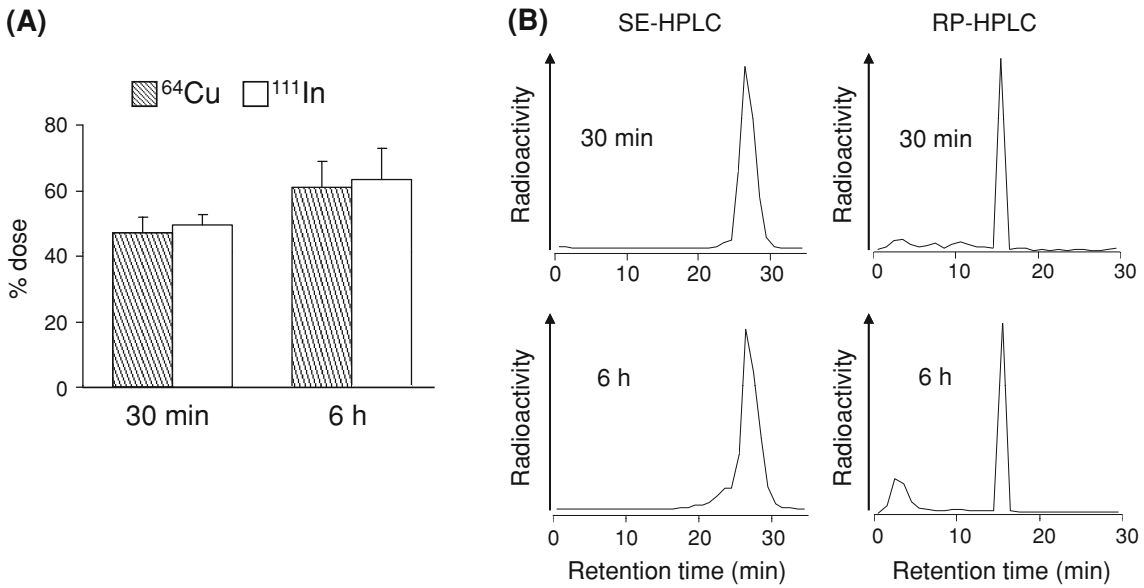

Fig. 3 Radioactivity analysis in the urine after administration of ^{64}Cu-DOTA-TOC and ^{111}In-DOTA-TOC. **a** Radioactivity of ^{64}Cu and ^{111}In excreted into the urine at 30 min and 6 h after administration of mixed solution of ^{64}Cu-DOTA-TOC and ^{111}In-DOTA-TOC. Each value represents the mean % injected dose ± SD of three animals. **b** Radioactivity profiles of urine at 30 min and 6 h after the administration of ^{64}Cu-DOTA-TOC by SE-HPLC and RP-HPLC

Discussion

The final goal of this study was to do a preclinical study of ^{64}Cu-DOTA-TOC as a PET imaging agent for somatostatin receptor-positive tumors. ^{64}Cu-DOTA-TOC required no purification, since the radiolabeling yield was more than 95% with 200–300 MBq of ^{64}Cu. Furthermore, the radioactivity of ^{64}Cu-DOTA-TOC, produced using a small hospital-installed cyclotron, would be sufficient for use in clinical studies, since Anderson et al. [4] showed that clear PET images of patients with neuroendocrine tumors were obtained with a 107–130 MBq injection of ^{64}Cu-TETA-OC.

In contrast to the high in vitro stability, the transchelation of ^{64}Cu to protein, slightly lower size than that of BSA, was observed in vivo as described previously [11, 12]. In a biodistribution study, ^{64}Cu-DOTA-TOC showed retention in the blood after 1 h and high accumulation in the liver and intestine, the same results as previously described [17]. Since liver is the critical organ involved in the regulation of copper homeostasis [18], ^{64}Cu transchelated to protein might accumulate in the liver and result in biliary excretion. Recently, a combined PET/CT system has been developed that provides detailed morphological information [19]. Therefore, accumulation in nontarget organs is not so critical, but high tumor accumulation is the most important property for the development of PET tracer. In U87MG tumor-bearing mice, ^{64}Cu-DOTA-TOC showed high accumulation and retention in the tumor, and the tumor-to-blood and tumor-to-muscle ratios reached 8.81 ± 1.17 and 45.1 ± 12.5 at 6 h after administration, respectively, indicating that ^{64}Cu-DOTA-TOC would be a potential PET tracer for imaging of somatostatin receptor-positive tumors.

In comparison studies with ^{64}Cu-TETA-OC, ^{64}Cu-DOTA-TOC showed significantly higher accumulations than ^{64}Cu-TETA-OC in the blood, liver, kidney, and intestine, since the Cu-DOTA complex undergoes more transchelation than the Cu-TETA complex [12]. On the other hand, the accumulation of ^{64}Cu-DOTA-TOC in the tumors was also significantly higher than that of ^{64}Cu-TETA-OC, and the tumor-to-organ ratio of ^{64}Cu-DOTA-TOC was almost the same as that of ^{64}Cu-TETA-OC in all organs except for muscle. It was reported that ^{111}In-labeled or ^{99m}Tc-labeled TOC showed higher accumulation in the tumor than that of ^{111}In-labeled or ^{99m}Tc-labeled OC, respectively [20, 21]. So, due to the high affinity toward somatostatin receptor, ^{64}Cu-DOTA-TOC accumulated to a high level in the tumor before transchelation occurred. Although comparison studies are needed in humans, ^{64}Cu-DOTA-TOC is potentially useful for PET imaging of tumors instead of ^{64}Cu-TETA-OC.

^{64}Cu-DOTA-TOC PET images showed the tumors very clearly, comparable to ^{18}F-FDG PET, indicating the possibility of its use in clinical studies. Interestingly, tumor accumulation of ^{64}Cu-DOTA-TOC was heterogeneous despite the relatively uniform accumulation of ^{18}F-FDG, which might provide valuable information about the characteristics of individual tumors. Because of the lower spatial resolution of SPECT compared to PET, the

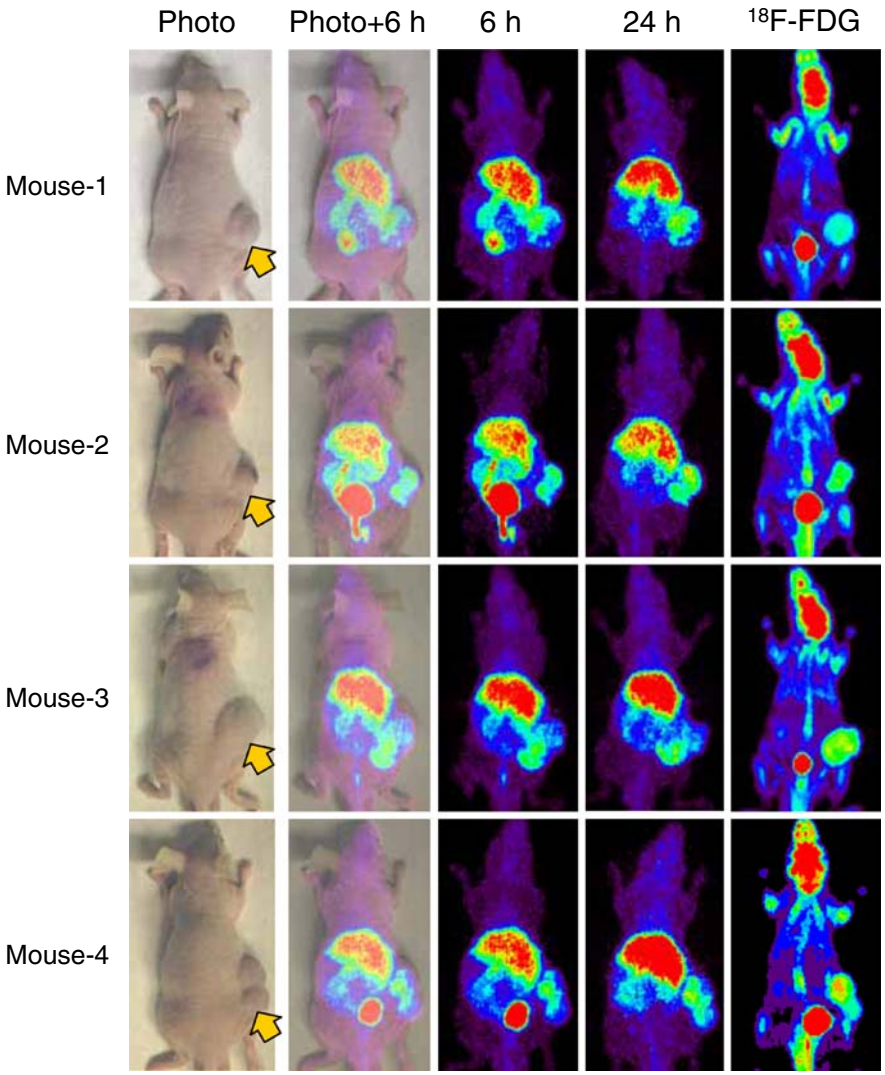

Fig. 4 PET images of U87MG tumor-bearing mice with ^{64}Cu-DOTA-TOC or ^{18}F-FDG. Four mice were imaged at 6 and 24 h after administration of ^{64}Cu-DOTA-TOC and at 1 h after administration of ^{18}F-FDG. Photographs (Photo) of mice were taken just before 6 h imaging of ^{64}Cu-DOTA-TOC and superimposed on corresponding PET image (Photo + 6 h). Arrows point to flank tumors

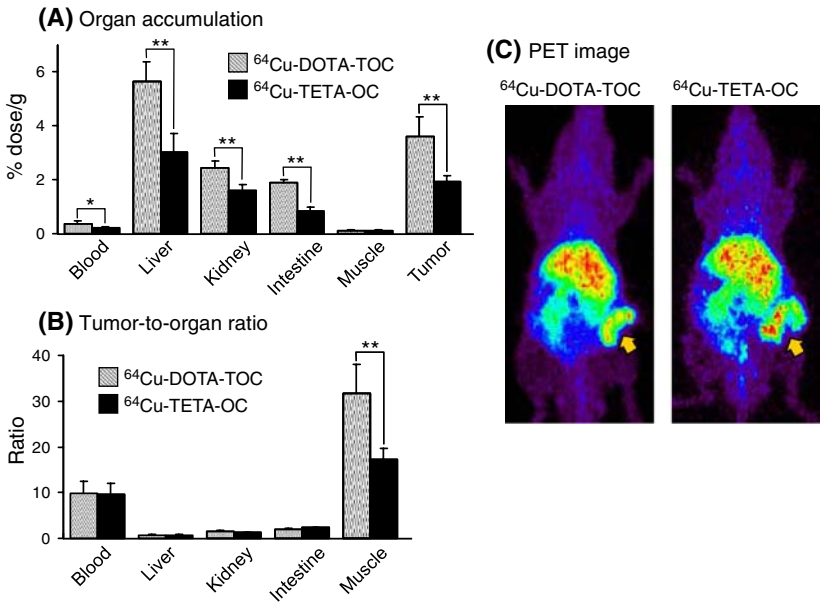

Fig. 5 Comparison of biodistribution and PET image between ^{64}Cu-DOTA-TOC and ^{64}Cu-TETA-OC in U87MG tumor-bearing mice. **a** % Injected dose/g of organ and **b** tumor-to-organ ratio at 6 h after administration of ^{64}Cu-DOTA-TOC or ^{64}Cu-TETA-OC. Each column represents 4 mice, and significant differences were determined ($*p < 0.05$, $**p < 0.005$). Mean tumor weight was 218 mg. **c** PET images were performed at 6 h after administration of ^{64}Cu-DOTA-TOC or ^{64}Cu-TETA-OC (not the same mouse)

approved radiolabeled octreotide analog, ^{111}In-DTPA-OC, would not be able to provide such information. Furthermore, ^{64}Cu-DOTA-TOC PET would be more useful than ^{111}In-DTPA-OC SPECT for choosing the appropriate case of therapy with non-radiolabeled or radiolabeled octreotide analogs. The ^{64}Cu-DOTA-TOC PET image was very similar to that of ^{64}Cu-TETA-OC. Since clear PET images of patients with neuroendocrine tumors were obtained with ^{64}Cu-TETA-OC [4], ^{64}Cu-DOTA-TOC is a potential PET tracer in the clinical phase.

^{64}Cu-DOTA-TOC showed steady clearance from the kidney, as previously described [17], and was also reported in case of other ^{64}Cu-labeled peptide [22–24]. Therefore, ^{64}Cu and ^{67}Cu would be suitable radionuclides for the therapy, since renal clearance could reduce renal toxicity, which is the major problem of radionuclide therapy using radiolabeled peptides or small proteins. Since the levels of radioactivity of ^{64}Cu and ^{111}In excreted in the urine were almost equal, ^{64}Cu would be released into the circulation from the kidney. One of the key copper-binding proteins, superoxide dismutase (SOD), is highly distributed in the kidney cytosol [18], leading to the hypothesis that ^{64}Cu is transchelated to the SOD in kidney cytosol, released into the blood, accumulated in the liver, and finally excreted in feces. Although the radioactivity levels of blood and liver were slightly high, they were much lower than radiolabeled antibodies, which have been efficiently used in the clinical phase as a treatment for malignant lymphoma [25, 26].

Conclusion

^{64}Cu-DOTA-TOC was prepared in high radiochemical yield sufficient for clinical practice. ^{64}Cu-DOTA-TOC showed high levels of accumulation in tumors and clear PET images in U87MG tumor-bearing mice. These findings indicated that ^{64}Cu-DOTA-TOC is a potential PET tracer for imaging somatostatin receptor-positive tumors in the clinical phase.

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