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[¹⁷⁷Lu]-DOTA⁰-Tyr³-octreotate: A Potential Targeted Radiotherapeutic for the Treatment of Medulloblastoma

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Abstract

Medulloblastoma, the most common pediatric brain tumor, is difficult to treat because conventional therapeutic approaches result in significant toxicity to normal central nervous system tissues, compromising quality of life. Given the fact that medulloblastomas express the somatostatin subtype 2 receptor, [¹⁷⁷Lu-DOTA⁰,Tyr³]octreotate ([¹⁷⁷Lu]DOTA-TATE) could be a potentially useful targeted radiotherapeutic for the treatment of this malignancy. The current study was undertaken to evaluate this possibility in preclinical models of D341 MED human medulloblastoma by comparing the properties of [¹⁷⁷Lu]DOTA-TATE to those of glucose-[¹²⁵I]-Tyr³]-octreotate ([¹²⁵I]Gluc-TOCA), a radiopeptide previously shown to target this cell line. *In vitro* assays indicated that both labeled peptides exhibited similar cell-associated and internalized radioactivity after a 30-min incubation at 37°C; however, at the end of the 4 h incubation period, the internalized radioactivity for [¹⁷⁷Lu]DOTA-TATE (6.22 ± 0.75%) was nearly twice that for [¹²⁵I]Gluc-TOCA (3.16 ± 0.27%), with similar differences seen in total cell-associated radioactivity levels. Consistent with the results from the internalization assays, results from paired-label tissue distribution studies in athymic mice with subcutaneous D341 MED medulloblastoma xenografts showed a similar degree of tumor accumulation for [¹⁷⁷Lu]DOTA-TATE and [¹²⁵I]Gluc-TOCA at early time points but by 24 h, a more than 5-fold advantage was observed for the ¹⁷⁷Lu-labeled peptide. Tumor-to-normal tissue ratios generally were more favorable for [¹⁷⁷Lu]DOTA-TATE at all time points, due in part to its lower accumulation in normal tissues except kidneys. Taken together, these results suggest that [¹⁷⁷Lu]DOTA-TATE warrants further investigation as a targeted radiotherapeutic for medulloblastoma treatment.

Keywords

Medulloblastoma; Lutetium-177; DOTA-TATE; Targeted Radiotherapy

INTRODUCTION

Medulloblastoma is the most common pediatric malignant brain tumor, with its highest incidence found in children between the ages of 3 and 9 years old [1]. Although aggressive treatment protocols have resulted in improvements in overall survival, particularly for patients with nondisseminated disease, the quality of life of long term survivors has frequently been severely compromised. Several studies have documented severe impairment of neurocognitive function, which are most apparent in children less than 4 years in age, and

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have been attributed to whole brain and craniospinal axis irradiation [2]. Thus, while conventional radiation therapy can be effective in controlling medulloblastoma, the neurologic and cognitive sequelae that results from its nonspecific application within the neuroaxis is a major limitation of this treatment method. One approach for improving the treatment of medulloblastoma with radiation is to refine the anatomical specificity of the radiation field exemplified by the use of a proton beam [3]. Another approach is to refine the molecular specificity of the radiation field through the use of targeted radionuclide therapy.

Particularly for potential applications in pediatric patients, the translational pathway for a targeted radiotherapeutic is simplified if the molecular target, delivery vehicle and radionuclide have been utilized previously in other patient populations. With regard to the first component, one of the most widely investigated areas of targeted therapy clinical research has involved molecules that bind selectively to somatostatin receptors (SSTR) that are abundantly expressed on a variety of neuroendocrine tumors [4,5]. Fortunately, the expression of SSTR at high densities also has been documented in medulloblastomas [6-10]. Although the number of samples generally was small, high level expression of SSTR (predominantly as subtype-2) was demonstrated on medulloblastoma biopsies and human cell lines by reverse transcription-PCR, Southern blot analysis, receptor autoradiography, immunohistochemistry and competitive membrane binding assays. Finally, ^{111}In -labeled pentetreotide (Octreoscan[®]) has been evaluated recently for somatostatin receptor imaging of children with medulloblastoma [11,12].

With regard to the selection of carrier molecule and radionuclide, the vast majority of studies evaluating the imaging and treatment of SSTR-expressing malignancies have utilized peptides as the delivery vehicle in combination with a wide variety of radionuclides. Much of this work was performed with octreotide, an octapeptide that is a metabolically stable analogue of the natural SSTR ligand somatostatin-14, which was derivatized with chelating agents such as DTPA and DOTA to permit labeling with radiometals such as ^{111}In and ^{90}Y [13]. Octreotate, an analogue of octreotide obtained by replacing its C-terminus residue threonol with threonine, is generally believed to have better biological properties than octreotide itself, in part because of its more rapid clearance from normal tissues [14-17]. Finally, analogues in which the phenylalanine at position 3 has been replaced with tyrosine (Tyr³-octreotide; TOC and Tyr³-octreotate; TOCA) have been developed to make them amenable for radioiodination.

Now that ^{177}Lu has become readily available, this radionuclide is increasingly used for targeted therapy because of certain advantages that it has in comparison with ^{131}I and ^{90}Y [18]. This is exemplified by the recent emergence of [^{177}Lu -DOTA⁰,Tyr³]octreotate as the peptide of choice for the clinical treatment of SSTR-expressing tumors such as gastroenteropancreatic cancers [19-22]. An important reason for this trend is that ^{177}Lu , like ^{131}I , emits low energy β -particles; however, its γ -emissions are of considerably lower energy (113 and 208 keV) and abundance (7% and 11%) than those of ^{131}I . Benefits of the more favorable γ -ray spectrum of ^{177}Lu include less stringent radiation protection requirements for patients and personnel, improved quality and quantification of dosimetric images, shorter periods of patient confinement, and lower collateral radiation dose deposition to normal tissues. The last two advantages would be of particular value in pediatric patients such as those with medulloblastoma, where minimizing isolation from family members and avoiding bystander irradiation of normal central nervous system tissues are critical considerations.

The current study was performed to evaluate the tissue distribution of [^{177}Lu -DOTA⁰,Tyr³]octreotate ([^{177}Lu]DOTA-TATE) in athymic mice with human medulloblastoma xenografts as an initial indicator of the potential utility of this labeled

peptide for treatment of children with this malignancy. Because we have previously demonstrated that glucose- $[^{131}\text{I-Tyr}^3]$ -octreotate ($[^{131}\text{I}]\text{Gluc-TOCA}$) possessed the best medulloblastoma targeting of the four radioiodinated peptides that were evaluated [17], experiments were performed in paired-label format with $[^{131}\text{I}]\text{Gluc-TOCA}$ and $[^{177}\text{Lu}]\text{DOTA-TATE}$ to determine which peptide should be moved forward towards clinical evaluation as a targeted radiotherapeutic for children with medulloblastoma.

EXPERIMENTAL PROCEDURES

General

Lutetium-177 was obtained from the University of Missouri as a solution in 0.05 N HCl at a concentration of 1.2-1.5 Ci/ml and with a specific activity of about 25 Ci/mg. Sodium $[^{125}\text{I}]$ iodide with a specific activity of 2200 Ci/mmol was purchased from Perkin-Elmer Life and Analytical Sciences (Boston, MA). DOTA- $[\text{Tyr}^3]$ -octreotate was obtained from Biosynthema (St. Louis, MO) and had a purity of 99.2%. Iodine-125 labeled Gluc-TOCA was synthesized as reported before [17]. High-pressure liquid chromatography (HPLC) was performed using a Beckman Gold HPLC system equipped with a Model 126 programmable solvent module, a Model 166 NM variable wavelength detector, a Model 170 radioisotope detector and a Beckman System Gold remote interface module SS420X; data were acquired using 32 Karat[®] software. Reversed-phase HPLC was performed using a 4.6×250 mm XTerra RP18 (5 : m) column eluted at 1 ml/min with a gradient of solvents consisted of 0.1% TFA in each water (solvent A) and acetonitrile (solvent B). The composition of B was increased linearly from 0% to 60% over a period of 30 min. Radio- thin layer chromatography (TLC) and paper chromatography (PC) were analyzed initially using a System 200 Imaging Scanner (BioScan, Washington, DC), and then the sheets were cut into strips and counted using an automated gamma counter (LKB 1282, Finland or Perkin-Elmer 1480 Wizard3, Turku, Finland).

Cells and culture conditions

D341 MED is a continuous cell line derived from a tumor biopsy of a patient with a cerebellar medulloblastoma [23]. These cells were grown in 10% FCS and zinc Option medium, and were maintained in a humidified atmosphere (37°C, 5% CO₂).

Synthesis of $[^{177}\text{Lu}]\text{-DOTA-TATE}$

The method for this synthesis was adapted from procedures reported in the literature [20,24-26]. Briefly, the DOTA-TATE peptide (10-100 : g) dissolved in 100 : 1 of 25 mM sodium ascorbate in 50 mM sodium acetate, pH 5.0 was added to a plastic vial containing an equal volume of ^{177}Lu (3-22 mCi) in 0.05 N HCl. The vial was sealed and heated at 80°C in a preheated heating block for 20 min. The cooled reaction mixture was diluted with phosphate-buffered saline (PBS). Radiochemical purity was determined by instant thin layer chromatography [20], reversed-phase thin layer chromatography [26], paper chromatography [25], and/or by reversed-phase HPLC. Under the conditions of HPLC (see above), the labeled peptide had a retention time of 20-21 min.

Internalization of $[^{125}\text{I}]\text{Gluc-TOCA}$ and $[^{177}\text{Lu}]\text{-DOTA-TATE}$

About 250,000 cpm (205 nCi) of $[^{125}\text{I}]\text{Gluc-TOCA}$ and 25,000 cpm (330 nCi) of $[^{177}\text{Lu}]\text{-DOTA-TATE}$ individually (single label) was incubated at 37°C in triplicate for 30 min, or 1, 2, 3, or 4 h with 1×10^6 D341 MED cells in tubes containing 1 ml of Zinc option medium. In parallel, incubations were performed in the presence of 1 : M octreotide to ascertain nonspecific uptake. To determine the intracellular activity, the surface-bound radioactivity was stripped off by incubation with 1 ml of 20 mM sodium acetate in Hank's buffered salt

solution, HBSS (pH 5.0) for 10 min at 37°C. At the end of this the incubation, the cells were washed twice with ice-cold Zinc option medium. After removing the supernatant, the cells were washed once with HBSS (pH 5.0), solubilized in 1 N NaOH, and counted for radioactivity in an automated gamma counter.

Paired-label biodistribution of [¹²⁵I]Gluc-TOCA and [¹⁷⁷Lu]DOTA-TATE in athymic mice bearing D341 Med xenografts

Animal studies were performed under the guidelines established by the Duke University Institutional Animal Care and Use Committee. Athymic BALB/c-*nu*(SPF) mice weighing 18–27 g, obtained from the breeding colony maintained at the Duke University Comprehensive Cancer Center Isolation Facility, were used in these studies. The D341 MED cell line was maintained *in vivo* by serial passage in athymic mice, with passage number 89 being used in these studies. Tumors were transplanted into the right flank with an inoculation volume of 50 : 1 and biodistribution studies were initiated when tumors were about 200–500 mm³. Mice received 3 : Ci each of [¹²⁵I]I-Gluc-TOCA and [¹⁷⁷Lu]DOTA-TATE *via* the tail vein, and groups of 5 animals were killed at 30 min, and 1 h, 4h, and 24 h after injection of labeled peptides. To determine the specificity of uptake in tumor and normal organs including those known to express SSTR, another group of 5 mice simultaneously was co-injected with 100 : g of octreotide and biodistribution performed 30 min later. Mice were killed with an overdose of isofluorane, dissected, and tissues of interest were isolated. Blot-dried tissues were weighed and counted along with dose standards. From these data, the uptake in different organs was calculated as the percent of injected dose per gram of tissue. A paired Student's *t* test was used to determine the statistical significance of the difference in the uptake of the two isotopes. For determining the statistical significance of the difference in the uptake between the controls and the octreotide-treated animals, an unpaired *t* test was used.

RESULTS AND DISCUSSION

More effective and less debilitating therapeutic approaches are needed for the treatment of medulloblastoma, the most common malignant tumor of the central nervous system in children. Targeted radiotherapy is attractive for this purpose because of the potential for focusing cytotoxic effects on malignant tissue. With medulloblastoma, 30-40% of patients recur either at the original tumor site or within the cerebrospinal fluid compartment [27], making it important to devise strategies for delivering therapeutic doses to tumor cells spread along the spinal cord without compromising normal tissues of the central nervous system. The feasibility of treating tumor within the leptomeningeal compartment in children with a targeted radiotherapeutic has been demonstrated by Kramer *et al* [28]. Iodine-131 labeled anti-GD2 monoclonal antibody was administered intrathecally to 15 children with neuroblastoma and significant prolongation in survival was observed with acceptable toxicity. The application of this tactic to medulloblastoma has been described in a case report [29]; an 8 year old boy with recurrent medulloblastoma was given 4 cycles of [⁹⁰Y-DOTA⁰,Tyr³]octreotate at monthly intervals and was in complete remission for 3 years. Although this result is encouraging, modeling studies indicate that the treatment of leptomeningeal tumor would be best accomplished with a shorter range β-emitter such as ¹³¹I or ¹⁷⁷Lu [30].

With that goal in mind, we previously evaluated 4 radioiodinated SSTR avid peptides in a series of experiments in human medulloblastoma models and [¹³¹I]Gluc-TOCA was demonstrated to be the best peptide in terms of maximizing internalized radioactivity *in vitro* and providing the highest and most selective tumor uptake *in vivo* [17]. On this basis, [¹³¹I]Gluc-TOCA was selected for direct comparison to [¹⁷⁷Lu]DOTA-TATE in the paired-label biodistribution experiments reported herein. In that way, it should be possible to factor

out the effects of differences in variables such as receptor number, tumor hemodynamics, and catabolism that can vary within groups of experimental animals. Slight modification of methods reported in the literature for the synthesis of [^{177}Lu]DOTA-TATE delivered the labeled peptide with a consistent incorporation yield of 95-99% ($n = 5$). A typical HPLC profile of the reaction mixture is shown in Figure 1; similar results were obtained using other analytical techniques including instant thin layer chromatography, reversed-phase thin layer chromatography, and paper chromatography. The specific activity of the [^{177}Lu]DOTA-TATE used in the biological assays was in the range of 600 to 900 $\mu\text{Ci}/\text{g}$, similar to the values reported by other investigators [20,26].

With peptide targeted radiotherapy, internalization of the labeled molecule into cancer cells after receptor binding is generally required to prolong tumor residence time and provide a good therapeutic index. In addition, the relative degree of trapping of radioactivity in the intracellular compartment measured *in vitro* for a series of SSTR-avid peptides can be predictive of their relative level of accumulation in tumor xenografts [15,17]. Earlier we have shown that, among a group of radioiodinated octreotide analogues, [^{125}I]Gluc-TOCA exhibited the highest tumor retention *in vitro* in D341 MED cells [17]. In the current study, the total cell-associated and intracellular retention of radioactivity from [^{177}Lu]DOTA-TATE after its internalization by D341 MED cells was compared with that from [^{125}I]Gluc-TOCA (Figure 2). The cell-associated and internalized radioactivity for both radiolabeled peptides was quite similar after a 30-min incubation at 37°C; however, the value of these parameters remained constant for the radioiodinated peptide but increased for the ^{177}Lu -labeled peptide. At the end of the 4 h incubation period, the internalized radioactivity for [^{177}Lu]DOTA-TATE (6.22 $\pm$ 0.75%) was nearly twice that for [^{125}I]Gluc-TOCA (3.16 $\pm$ 0.27%), with similar differences seen in total cell-associated activity levels.

Although the internalization of DOTA-TATE labeled with other radionuclides in other cell lines has been investigated [31,32], to our knowledge, there is only one study in which the internalization of [^{177}Lu]DOTA-TATE has been evaluated [14]. Capello *et al.* [14] have reported that about 0.03% of [^{177}Lu]DOTA-TATE dose was internalized after 1 h of incubation with 1×10^5 CA20948 rat pancreatic tumor cells. In the current study, the fraction of [^{177}Lu]DOTA-TATE dose that was internalized by 1×10^6 D341 MED cells was little more than 6% of the input dose at 4 h. Although this is more than about 20-fold higher than that reported for CA20948 cells by Capello *et al.*, these values were considerably lower than the up to 20-25% levels reported for other analogues in different cell lines [32,33]. However, the magnitude of intracellularly trapped radioactivity for [^{125}I]Gluc-TOCA in the paired-label assay also was considerably lower than these levels (Figure 2). Although up to about 8-14% intracellular trapping has been seen with radiolabeled [^{125}I]Gluc-TOCA in D341 MED cells [17,34,35], considerably lower levels have also been observed in previous studies [36]. One factor to which these differing results had been attributed is the use of differing passage numbers of the cell line. Another possibility is that the lower internalized levels observed in this study were due to the presence of unlabeled peptide in the incubation medium. The [^{125}I]Gluc-TOCA used in the internalization assay was purified by HPLC and thus, should have a specific activity approximating that of the radioiodine, which was ~ 2200 Ci/mmol. On the other hand, in the synthesis of [^{177}Lu]DOTA-TATE, the starting peptide DOTA-TATE was not separated from the final preparation. The total peptide concentration in each case in the *in vitro* assay was about 0.1 nM. Several investigators have shown that the presence of unlabeled peptide can decrease the cellular uptake and internalization of labeled octreotide analogues both *in vitro* and *in vivo* [14,37-39]; however, the opposite behavior has been reported for the internalization of [^{125}I]I-TOC in the presence of unlabeled octreotide concentrations of less than 1 nM [39]. Finally, a recent study utilizing both quantitative fluorescent activated cell sorting and Scatchard analysis has indicated that D341 MED cells have an average of about 4×10^5 SSTR per cell [40], and thus the

internalization assay was performed in receptor excess. For these reasons, it is unlikely that the presence of unlabeled peptide contributed to the relatively low uptake and internalization of the labeled peptides in these experiments.

The tissue distribution of ^{177}Lu and ^{125}I activity after intravenous injection of [^{177}Lu]DOTA-TATE and [^{125}I]Gluc-TOCA was determined in athymic mice with subcutaneous D341 MED human medulloblastoma xenografts. By performing the experiment in paired-label format, the relative uptake of the two labeled molecules could be compared in the same animals. The percentage injected dose per gram (% ID/g) recovered from tumor and normal tissues of ^{177}Lu and ^{125}I is summarized in Table 1. Tumor accumulation of the two radiolabeled peptides were not significantly different from each other at 30 min, 1 h and 4 h; however, at 24 h, the $^{177}\text{Lu}/^{125}\text{I}$ activity ratio was 6.5 ± 0.5 , indicating a significant advantage in tumor retention for [^{177}Lu]DOTA-TATE. The tumor uptake value observed for the uptake of [^{177}Lu]DOTA-TATE in D341 MED human medulloblastoma xenografts is within the same range reported for this targeted therapeutic in mice with other types of human tumor xenografts. At 24 h, the only common time point available for comparison, our current results of $9.1 \pm 5.2\%$ ID/g is intermediate between those reported for mice with small cell lung cancer ($3.7 \pm 1.0\%$ ID/g) [41,42] and those with carcinoid ($16.0 \pm 1.4\%$ ID/g) [16] xenografts. One can only speculate that the variation in tumor levels observed among the different xenografts likely reflects the influence of factors such as tumor hemodynamics, receptor number and subtype spectrum, as well as tumor size.

The retention of radioactivity in normal tissues that do not express SSTR such as the liver, muscle and blood was significantly lower ($p < 0.05$) for the ^{177}Lu -labeled peptide from 30 min to 4 h with the opposite behavior seen at the 24 h time point. A notable exception is the kidneys, where $^{177}\text{Lu}/^{125}\text{I}$ activity ratios increased from 1.2 ± 0.1 at 30 min to 6.3 ± 1.4 at 24 h. In pancreas and lungs, organs known to express somatostatin receptors, the uptake of the ^{177}Lu -labeled peptide was significantly higher than that of the ^{125}I -labeled peptide, particularly at later time points. Although the stomach also expresses SSTR, comparisons are less clear due to the potential for free iodide to also accumulate in this organ. Lower uptake of ^{125}I in SSTR-positive tissues such as tumor and pancreas and higher uptake in other tissues may partly be due to more rapid metabolism of the radioiodinated peptide. Petrik *et al.* [43] have compared the metabolism of [^{125}I]I-Gluc-TOCA and [^{177}Lu]DOTA-TATE in rats. While predominantly intact form of [^{177}Lu]DOTA-TATE was present in urine, the radioiodinated analogue underwent extensive metabolism. As is typical with radiometallated radiopharmaceuticals, kidney uptake throughout and bone uptake at later time points for [^{177}Lu]DOTA-TATE were higher.

To determine the specificity of radio-peptide uptake, the tissue distribution of [^{177}Lu]DOTA-TATE and [^{125}I]Gluc-TOCA accumulation also was determined 30 min after co-administration with a blocking dose of 100 μg of octreotide. As shown in Figure 3, the SSTR-specific nature of peptide accumulation in D341 MED xenografts was confirmed by the reduction of [^{177}Lu]DOTA-TATE and [^{125}I]Gluc-TOCA uptake to $8 \pm 3\%$ and $12 \pm 5\%$ of control levels, respectively, in the presence of a blocking dose of cold octreotide. Similar effects were seen in stomach and pancreas and to a lesser extent, the lungs; however, in all tissues, the degree of blocking that was achieved was greater for the ^{177}Lu -labeled peptide.

An important metric for determining the potential utility of a labeled compound as a targeted radiotherapeutic is the selectivity of tumor localization, which is generally expressed as the tumor-to-normal tissue ratio. Figure 4 summarizes the tumor-to-normal tissue ratios observed for both labeled peptides. More favorable contrast in uptake between tumor and normal tissues was generally observed, except in the kidneys, with [^{177}Lu]DOTA-TATE. For example, at 1 h after injection tumor-to-normal tissue ratios for blood, liver, kidney,

spleen, brain and muscle for [^{177}Lu]DOTA-TATE were 142:1, 35:1, 5:1, 47:1, 1063:1 and 176:1, respectively, compared with values of 39:1, 14:1, 8:1, 37:1, 477:1 and 112:1, respectively, for [^{125}I]Gluc-TOCA.

In conclusion, [^{177}Lu]DOTA-TATE exhibited excellent accumulation in the D341 MED human medulloblastoma xenograft and high tumor-to normal tissue ratios. Because ^{177}Lu -labeled peptide exhibited more favorable localization characteristics than co-administered [^{125}I]Gluc-TOCA, [^{177}Lu]DOTA-TATE has been selected for more extensive evaluation as a targeted radiotherapeutic for the treatment of medulloblastoma. Toxicity and therapeutic efficacy trials are underway to determine the feasibility of treating human medulloblastoma neoplastic meningitis in athymic rats with intrathecally administered [^{177}Lu]DOTA-TATE.

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Figure 1.
Typical HPLC profile of reaction mixture from the labeling of DOTA-TATE with ^{177}Lu
(see text for HPLC conditions).

Figure 2. Internalization of $[^{125}\text{I}]\text{Gluc-TOCA}$ and $[^{177}\text{Lu}]\text{DOTA-TATE}$ by D341 MED cells as a function of time. Cells (1×10^6) were incubated with each labeled peptide individually in the presence or absence of 1 :M octreotide for various time periods. After removing cell culture supernatants containing unbound radioactivity, the surface-bound radioactivity was stripped by treatment with an acidic medium. Radioactivity that was internalized and surface-bound was determined. Shown are the specific (total minus nonspecific) cell-associated (internalized + surface-bound; left panel) and internalized (right panel) radioactivity as percent of input dose.

Figure 3. Effect of the co-injection of unlabeled octreotide on the uptake of [^{125}I]Gluc-TOCA and [^{177}Lu]DOTA-TATE in tumor and SSTR-expressing tissues in athymic mice bearing D341 MED medulloblastoma xenografts at 30 min after injection.

Figure 4.

Tumor-to-normal tissue ratios obtained in athymic mice bearing D341 MED medulloblastoma xenografts after i.v. injection of [^{125}I]Gluc-TOCA (left panel) and [^{177}Lu]DOTA-TATE (right panel). Notice that the values for brain are multiplied by 0.1 to facilitate their display.

Table 1
Paired-label biodistribution of [¹²⁵I]Gluc-TOCA and [¹⁷⁷Lu]DOTA-TATE in athymic mice bearing D341 MED xenografts

Tissue	Percent injected dose per gram tissue ^a							
	0.5 h		1 h		4 h		24 h	
	Gluc-TOCA	DOTA-TATE	Gluc-TOCA	DOTA-TATE	Gluc-TOCA	DOTA-TATE	Gluc-TOCA	DOTA-TATE
Liver	2.13 ± 0.55	0.87 ± 0.20	1.73 ± 0.20	0.69 ± 0.10 ^b	0.64 ± 0.25	0.42 ± 0.11	0.07 ± 0.04	0.21 ± 0.06
Spleen	1.20 ± 0.33	1.02 ± 0.42 ^b	1.33 ± 1.26	1.37 ± 1.52 ^b	0.60 ± 0.16	0.51 ± 0.13	0.05 ± 0.01	0.21 ± 0.05
Lungs	5.66 ± 1.79	7.41 ± 2.20 ^b	2.84 ± 1.99	3.17 ± 2.92 ^b	2.90 ± 1.77	4.12 ± 2.72	0.13 ± 0.07	2.19 ± 1.18
Heart	0.63 ± 0.09	0.42 ± 0.08	0.50 ± 0.16	0.30 ± 0.14	0.34 ± 0.09	0.14 ± 0.04	0.03 ± 0.01	0.08 ± 0.02
Kidneys	4.19 ± 1.19	5.02 ± 1.59 ^b	3.34 ± 0.61	5.10 ± 0.93	1.76 ± 0.43	3.55 ± 1.00	0.23 ± 0.11	1.34 ± 0.26
Stomach	6.70 ± 2.12	6.76 ± 2.06 ^b	11.77 ± 2.77	11.42 ± 2.68 ^b	7.30 ± 2.76	5.00 ± 1.63	0.30 ± 0.22	1.36 ± 0.07
Sm. Intestine	13.13 ± 3.15	3.32 ± 0.98	14.47 ± 3.44	4.76 ± 0.58	3.74 ± 0.74	2.36 ± 0.17	0.08 ± 0.03	0.51 ± 0.07
Lg. Intestine	2.00 ± 0.38	2.38 ± 0.44 ^b	12.56 ± 6.40	4.12 ± 0.52	23.06 ± 4.83	7.03 ± 1.79	0.29 ± 0.05	1.25 ± 0.11
Muscle	0.33 ± 0.07	0.35 ± 0.12 ^b	0.24 ± 0.05	0.16 ± 0.06	0.20 ± 0.06	0.09 ± 0.06	0.01 ± 0.01	0.04 ± 0.02
Blood	1.28 ± 0.29	0.60 ± 0.14	0.77 ± 0.28	0.22 ± 0.12	0.52 ± 0.21	0.12 ± 0.08	0.02 ± 0.01	0.03 ± 0.01 ^b
Bone	0.86 ± 0.24	0.91 ± 0.40 ^b	0.66 ± 0.20	0.51 ± 0.36	0.37 ± 0.06	0.49 ± 0.09	0.02 ± 0.02	0.46 ± 0.18
Brain	0.11 ± 0.08	0.13 ± 0.18 ^b	0.06 ± 0.01	0.03 ± 0.01	0.07 ± 0.02	0.03 ± 0.01	0.00 ± 0.00	0.02 ± 0.01
Pancreas	28.18 ± 4.54	32.77 ± 4.56 ^b	20.82 ± 10.99	25.35 ± 13.60	6.07 ± 2.22	10.67 ± 2.62	0.21 ± 0.10	3.56 ± 1.16
Tumor	17.99 ± 7.17	18.88 ± 6.15 ^b	25.31 ± 15.33	24.26 ± 13.08 ^b	14.80 ± 4.61	14.89 ± 4.55 ^b	1.45 ± 0.94	9.12 ± 5.20

^aMean ± S.D. (n = 5).

^bThe difference in the uptake of the two tracers not significant ($p > 0.05$).