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Melanoma targeting property of a Lu-177-labeled lactam bridge-cyclized alpha-MSH peptide

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Abstract

The purpose of this study was to determine the melanoma targeting property of ¹⁷⁷Lu-DOTA-GGNle-CycMSH_{hex} in B16/F1 melanoma-bearing C57 mice. ¹⁷⁷Lu-DOTA-GGNle-CycMSH_{hex} exhibited high receptor-mediated melanoma uptake and fast urinary clearance. The tumor uptake of ¹⁷⁷Lu-DOTA-GGNle-CycMSH_{hex} was 20.25 ± 4.59 and 21.63 ± 6.27% ID/g at 0.5 and 2 h post-injection, respectively. Approximately 83% of injected dose cleared out the body via urinary system at 2 h post-injection. ¹⁷⁷Lu-DOTA-GGNle-CycMSH_{hex} showed high tumor to normal organ uptake ratios except for the kidneys. The tumor/kidney uptake ratios of ¹⁷⁷Lu-DOTA-GGNle-CycMSH_{hex} were 2.76 and 1.74 at 2 and 24 h post-injection. The melanoma lesions were clearly visualized by SPECT/CT using ¹⁷⁷Lu-DOTA-GGNle-CycMSH_{hex} as an imaging probe at 2 h post-injection. Overall, high melanoma uptake coupled with fast urinary clearance of ¹⁷⁷Lu-DOTA-GGNle-CycMSH_{hex} underscored its potential for melanoma treatment in the future.

Keywords

Alpha-melanocyte stimulating hormone; ¹⁷⁷Lu-labeled lactam bridge-cyclized peptide; melanoma targeting

Skin cancer is the most commonly diagnosed cancer in the United States, with approximately 3.5-million new cases occurring annually. Basal cell carcinoma, squamous cell carcinoma and melanoma are three major types of skin cancer. Malignant melanoma is the most lethal form of skin cancer, accounting for 75% of deaths of skin cancer despite the fact that melanoma only accounts for less than 5% of skin cancer cases.¹ High mortality of melanoma is tightly associated with metastatic melanoma which is resistant to current chemotherapy and immunotherapy. Thus, it is desirable to develop receptor-targeting peptide radiopharmaceuticals for melanoma imaging and therapy.^{2–21} At the present time, receptor-targeting radionuclide therapy represents a promising strategy for melanoma treatment. Melanocortin-1 (MC1) receptor-targeting alpha-melanocyte stimulating hormone (α-MSH) peptides have been utilized as delivering vehicles to target therapeutic radionuclides to melanoma cells for treatment.^{10,12,19} This strategy takes advantage of rapid distribution through blood circulation, receptor-targeting melanoma localization, and fast urinary clearance of radiolabeled α-MSH peptides.

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Over the past several years, we have identified a novel class of lactam bridge-cyclized α -MSH peptides through structure-activity-relationship studies for melanoma imaging.^{22–29} The MC1 receptor binding motif (His-DPhe-Arg-Trp) was cyclized by an Asp-Lys lactam bridge to yield the CycMSH_{hex} {c[Asp-His-DPhe-Arg-Trp-Lys]-CONH₂} peptide. The radiometal chelators of DOTA (1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid) and NOTA (1,4,7-triazacyclononane-1,4,7-triacetic acid) were coupled to the N terminus of the CycMSH_{hex} for SPECT (single photon emission computed tomography) and PET (positron emission tomography) imaging of melanoma.^{27–29} The melanoma lesions could be clearly visualized by SPECT using ¹¹¹In-DOTA-GGNle-CycMSH_{hex} or ⁶⁷Ga-NOTA-GGNle-CycMSH_{hex} as imaging probes,^{27,28} as well as by PET using ⁶⁴Cu-NOTA-GGNle-CycMSH_{hex} as an imaging probe.²⁹

Building upon the success of this novel class of lactam bridge-cyclized α -MSH peptides for melanoma imaging, we managed to extend their application to melanoma therapy using therapeutic radionuclides. We were particularly interested in ¹⁷⁷Lu due to its attractive decay properties. ¹⁷⁷Lu is medium-energy (0.497 MeV) β -emitter with a maximum soft tissue penetration of approximately 1.8–2 mm.³⁰ Such short penetration in soft tissue makes ¹⁷⁷Lu an ideal radioisotope for treating small tumors and metastases, where the therapeutic radiations stay fairly localized. Meanwhile, ¹⁷⁷Lu is a manageable radioisotope in terms of dose preparation and waste disposal due to its half-life of 6.71 days, and is commercially available. Moreover, ¹⁷⁷Lu emits γ -rays (113 and 208 keV) that are suitable for SPECT imaging. Thus, we prepared ¹⁷⁷Lu-DOTA-GGNle-CycMSH_{hex} and examined its cellular internalization and efflux properties in B16/F1 melanoma cells, and determined its melanoma targeting and pharmacokinetic properties in B16/F1 melanoma-bearing C57 mice in this study.

Firstly, DOTA-GGNle-CycMSH_{hex} was synthesized and purified by reverse phase high pressure liquid chromatography (RP-HPLC) according to our published procedure.²⁷ After the HPLC purification, DOTA-GGNle-CycMSH_{hex} displayed greater than 90% purity. The identity of DOTA-GGNle-CycMSH_{hex} was confirmed by electrospray ionization mass spectrometry. ¹⁷⁷Lu-DOTA-GGNle-CycMSH_{hex} (Figure 1) was readily prepared in 0.5 M ammonium acetate with greater than 95% radiolabeling yield, and was completely separated from its excess non-labeled peptide by RP-HPLC. The retention time of ¹⁷⁷Lu-DOTA-GGNle-CycMSH_{hex} was 17.8 min. ¹⁷⁷Lu-DOTA-GGNle-CycMSH_{hex} was stable in mouse serum at 37 °C for 24 h. Only ¹⁷⁷Lu-DOTA-GGNle-CycMSH_{hex} was detected by RP-HPLC after 24 h of incubation (Figure 2). Cellular internalization and efflux properties of ¹⁷⁷Lu-DOTA-GGNle-CycMSH_{hex} were examined in B16/F1 melanoma cells. Figure 3 illustrates the internalization and efflux of ¹⁷⁷Lu-DOTA-GGNle-CycMSH_{hex}. ¹⁷⁷Lu-DOTA-GGNle-CycMSH_{hex} exhibited rapid cellular internalization and prolonged cellular retention. Approximately 90% of ¹⁷⁷Lu-DOTA-GGNle-CycMSH_{hex} was internalized in the cells after 20 min of incubation. Cellular efflux results indicated that 40% of the ¹⁷⁷Lu-DOTA-GGNle-CycMSH_{hex} activity remained inside the cells at 2 h of incubation in the culture medium.

Secondly, the melanoma targeting and pharmacokinetic properties of ¹⁷⁷Lu-DOTA-GGNle-CycMSH_{hex} were determined in B16/F1 melanoma-bearing mice. The biodistribution results of ¹⁷⁷Lu-DOTA-GGNle-CycMSH_{hex} are presented in Table 1. ¹⁷⁷Lu-DOTA-GGNle-CycMSH_{hex} displayed rapid and high melanoma uptake. The tumor uptake was 20.25 ± 4.59 and 21.63 ± 6.27% ID/g at 0.5 and 2 h post-injection, respectively. ¹⁷⁷Lu-DOTA-GGNle-CycMSH_{hex} exhibited prolonged tumor retention, with 8.24 ± 1.51% ID/g of tumor uptake at 24 h post-injection. The co-injection of non-radioactive NDP-MSH blocked 96.3% of the tumor uptake, demonstrating that the tumor uptake was MC1 receptor-mediated. Whole-body clearance of ¹⁷⁷Lu-DOTA-GGNle-CycMSH_{hex} was rapid, with approximately 83% of the injected dose being washed out of the body via urinary system by 2 h post-injection.

Ninety-three percent of the injected dose cleared out of the body by 24 h post-injection. Normal organ uptake of ^{177}Lu -DOTA-GGNle-CycMSH_{hex} was generally low (<1.37% ID/g) at 2 h post-injection except for kidneys. High tumor/blood and tumor/normal organ uptake ratios were demonstrated as early as 0.5 h post-injection. The renal uptake was 13.83 ± 2.51 , 7.83 ± 1.38 , and $9.68 \pm 1.95\%$ ID/g at 0.5, 2 and 4 h post-injection, respectively. At 24 h post-injection, the kidney uptake was $4.75 \pm 1.03\%$ ID/g. The co-injection of NDP-MSH didn't reduce the renal uptake, indicating that the renal uptake of ^{177}Lu -DOTA-GGNle-CycMSH_{hex} was not receptor-mediated. The tumor/kidney uptake ratio was 2.76 and 1.74 at 2 and 24 h post-injection, respectively.

Over the past several years, several MC1 receptor-targeting ^{177}Lu -labeled metal-cyclized α -MSH peptides have been reported for melanoma therapy.^{14,15,19} Initially, the (Arg¹¹)CCMSH peptide was cyclized with non-radioactive Re to retain favorable melanoma targeting properties, whereas the DOTA was conjugated to the N-terminus of the peptide for ^{177}Lu labeling.¹⁴ ^{177}Lu -DOTA-Re(Arg¹¹)CCMSH exhibited 14.48 ± 0.85 and $17.68 \pm 3.32\%$ ID/g of tumor uptake at 2 and 4 h post-injection in B16/F1 melanoma-bearing C57 mice. The renal uptake of ^{177}Lu -DOTA-Re(Arg¹¹)CCMSH was $17.99 \pm 2.47\%$ and $19.09 \pm 2.38\%$ ID/g at 2 and 4 h post-injection in B16/F1 melanoma-bearing C57 mice. Furthermore, the introduction of Glu² between the DOTA and (Arg¹¹)CCMSH significantly decreased the renal uptake of ^{177}Lu -DOTA-Re(Glu², Arg¹¹)CCMSH by 70% as compared to ^{177}Lu -DOTA-Re(Arg¹¹)CCMSH at 2 and 4 h post-injection.¹⁵ Although the tumor uptake of ^{177}Lu -DOTA-Re(Glu², Arg¹¹)CCMSH was lower than that of ^{177}Lu -DOTA-Re(Arg¹¹)CCMSH, ^{177}Lu -DOTA-Re(Glu², Arg¹¹)CCMSH showed enhanced tumor/kidney uptake ratios than ^{177}Lu -DOTA-Re(Arg¹¹)CCMSH at 2 and 4 h post-injection.¹⁵ Remarkably, ^{177}Lu -DOTA-GGNle-CycMSH_{hex} exhibited higher tumor/kidney uptake ratios than ^{177}Lu -DOTA-Re(Glu², Arg¹¹)CCMSH at 0.5, 2 and 24 h post-injection. The tumor/kidney uptake ratios of ^{177}Lu -DOTA-GGNle-CycMSH_{hex} were 1.5, 1.5 and 1.8 times the tumor/kidney uptake ratios of ^{177}Lu -DOTA-Re(Glu², Arg¹¹)CCMSH at 0.5, 2 and 24 h post-injection, respectively.

The representative whole-body SPECT/CT images are presented in Figure 4. The B16/F1 melanoma lesions were clearly visualized by SPECT/CT using ^{177}Lu -DOTA-GGNle-CycMSH_{hex} as an imaging probe at 2 h post-injection. The imaging properties of ^{177}Lu -DOTA-GGNle-CycMSH_{hex} could potentially be utilized to calculate the absorbed dose of ^{177}Lu -DOTA-GGNle-CycMSH_{hex} treatment, as well as to monitor the therapeutic response without injecting an additional imaging probe. Furthermore, ^{177}Lu -DOTA-GGNle-CycMSH_{hex} exhibited high tumor to normal organ uptake ratios except for kidneys, suggesting that the kidneys would be dose-limiting normal organ for ^{177}Lu -DOTA-GGNle-CycMSH_{hex} treatment in the future. However, it is worthwhile to note that the renal uptake of ^{177}Lu -DOTA-GGNle-CycMSH_{hex} was lower than that of ^{177}Lu -DOTA-Re(Arg¹¹)CCMSH at all time points investigated in this study. The ^{177}Lu -DOTA-Re(Arg¹¹)CCMSH treatment (1×37.0 MBq or 2×18.5 MBq) significantly decreased the B16/F1 tumor growth and extended the mean survival time of B16/F1 tumor-bearing mice without any overt signs of renal toxicity.¹⁹ Accordingly, it is expected that same treatment doses of ^{177}Lu -DOTA-GGNle-CycMSH_{hex} would yield therapeutic effects without renal toxicity. Moreover, it is anticipated that the enhanced tumor/kidney uptake ratio of ^{177}Lu -DOTA-GGNle-CycMSH_{hex} would improve its therapeutic efficacy for melanoma. It would be interesting to determine the therapeutic efficacy of ^{177}Lu -DOTA-GGNle-CycMSH_{hex} in the future.

In conclusion, the tumor targeting and pharmacokinetic properties of ^{177}Lu -DOTA-GGNle-CycMSH_{hex} were determined in B16/F1 melanoma-bearing C57 mice in this study. Overall, the properties of high melanoma uptake and fast urinary clearance of ^{177}Lu -DOTA-GGNle-

CycMSH_{hex} underscored its potential as a therapeutic agent for metastatic melanoma detection in the future.

The experimental details are presented in References and notes.^{31–34}

Acknowledgments

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31. Peptide radiolabeling with ^{177}Lu : Amino acid and resin were purchased from Advanced ChemTech Inc. (Louisville, KY) and Novabiochem (San Diego, CA). DOTA-tri-*t*-butyl ester was purchased from Macrocyclics Inc. (Richardson, TX) for peptide synthesis. All other chemicals used in this study were purchased from Thermo Fischer Scientific (Waltham, MA) and used without further purification. DOTA-GGNle-CycMSH_{hex} was synthesized and characterized according to our published procedure.²⁷ $^{177}\text{LuCl}_3$ was purchased from Trace Life Sciences, Inc. (Dallas, TX) for peptide radiolabeling. ^{177}Lu -DOTA-GGNle-CycMSH_{hex} was prepared in a 0.5 M NH_4OAc -buffered solution at pH 5.4 according to the published procedure.¹⁹ Briefly, 50 μL of $^{177}\text{LuCl}_3$ (37–74 MBq in 0.05 M HCl aqueous solution), 10 μL of 1 mg/mL DOTA-GGNle-CycMSH_{hex} aqueous solution and 400 μL of 0.5 M NH_4OAc (pH 5.4) were added into a reaction vial and incubated at 75°C for 45 min. After the incubation, 10 μL of 0.5% ethylenediaminetetraacetic acid (EDTA) aqueous solution was added into the reaction vial to scavenge potential unbound $^{177}\text{Lu}^{3+}$ ions. The radiolabeled complexes were purified to single species by Waters RP-HPLC (Milford, MA) on a Grace Vydac C-18 reverse phase analytical column (Deerfield, IL) using a 20-minute gradient of 18–28% acetonitrile in 20 mM HCl aqueous solution with a flow rate of 1.0 mL/min. Purified peptide sample was purged with N_2 gas for 20 minutes to remove the acetonitrile. The pH of final solution was adjusted to 7.4 with 0.1 N NaOH and sterile normal saline for animal studies. *In vitro* serum stability of ^{177}Lu -DOTA-GGNle-CycMSH_{hex} was determined by incubation in mouse serum at 37 °C for 24 h and monitored for degradation by RP-HPLC.
32. Cellular internalization and efflux of ^{177}Lu -DOTA-GGNle-CycMSH_{hex}: B16/F1 murine melanoma cells were obtained from American Type Culture Collection (Manassas, VA). Cellular internalization and efflux of ^{177}Lu -DOTA-GGNle-CycMSH_{hex} were evaluated in B16/F1 melanoma cells. After being washed twice with binding medium [modified Eagle's medium with 25 mM *N*-(2-hydroxyethyl)-piperazine-*N'*-(2-ethanesulfonic acid), pH 7.4, 0.2% bovine serum albumin (BSA), 0.3 mM 1,10-phenanthroline], the B16/F1 cells seeded in cell culture plates were incubated at 25°C for 20, 40, 60, 90 and 120 min (n=3) in the presence of approximate 200,000 counts per minute (cpm) of HPLC-purified ^{177}Lu -DOTA-GGNle-CycMSH_{hex}. After incubation, the reaction medium was aspirated and the cells were rinsed with 2×0.5 mL of ice-cold pH 7.4, 0.2% BSA / 0.01 M PBS. Cellular internalization of ^{177}Lu -DOTA-GGNle-CycMSH_{hex} was assessed by washing the cells with acidic buffer [40 mM sodium acetate (pH 4.5) containing 0.9% NaCl and 0.2% BSA] to remove the membrane-bound radioactivity. The remaining internalized radioactivity was obtained by lysing the cells with 0.5 mL of 1N NaOH for 5 min. Membrane-bound and internalized ^{177}Lu -DOTA-GGNle-CycMSH_{hex} activities were counted in a gamma counter. Cellular efflux of ^{177}Lu -DOTA-GGNle-CycMSH_{hex} was determined by incubating the B16/F1 cells with ^{177}Lu -DOTA-Nle-CycMSH_{hex} for 2 h at 25°C, removing non-specific-bound activity with 2×0.5 mL of ice-cold PBS rinse, and monitoring radioactivity released into cell culture medium. At time points of 20, 40, 60, 90 and 120 min, the radioactivities on the cell surface and inside the cells were separately collected and counted in a gamma counter.
33. Biodistribution studies: All the animal studies were conducted in compliance with Institutional Animal Care and Use Committee approval. The pharmacokinetics of ^{177}Lu -DOTA-GGNle-CycMSH_{hex} was determined in B16/F1 melanoma-bearing C57 female mice (Harlan, Indianapolis, IN). Each C57 mouse was subcutaneously inoculated on the right flank with 1×10^6 B16/F1 cells. The weight of tumors reached approximately 0.2 g 10 days post cell inoculation.

Each melanoma-bearing mouse was injected with 0.037 MBq of ^{177}Lu -DOTA-GGNle-CycMSH_{hex} via the tail vein. Groups of 5 mice were sacrificed at 0.5, 2, 4 and 24 h post-injection, and tumors and organs of interest were harvested, weighed and counted. Blood values were taken as 6.5% of the whole-body weight. The tumor uptake specificity of ^{177}Lu -DOTA-GGNle-CycMSH_{hex} was determined by co-injecting 10 μg (6.07 nmol) of unlabeled NDP-MSH peptide with ^{177}Lu -DOTA-GGNle-CycMSH_{hex} at 2 h post-injection. Statistical analysis was performed using the Student's t-test for unpaired data. A 95% confidence level was chosen to determine the significance of difference in tumor and renal uptake of ^{177}Lu -DOTA-Nle-CycMSH_{hex} with/without NDP-MSH co-injection in the biodistribution studies described above. Differences at the 95% confidence level ($p < 0.05$) were considered significant.

34. Melanoma imaging with ^{177}Lu -DOTA-GGNle-CycMSH_{hex}: Approximately 17.4 MBq of ^{177}Lu -DOTA-GGNle-CycMSH_{hex} was injected in a B16/F1 melanoma-bearing C57 mouse (10 days post the cell inoculation) via the tail vein for melanoma imaging. The mouse was sacrificed for small animal SPECT/CT (Nano-SPECT/CT[®], Bioscan) imaging at 2 h post-injection. The 9-min CT imaging was immediately followed by the whole-body SPECT imaging. The SPECT scans of 24 projections were acquired. Reconstructed SPECT and CT data were visualized and co-registered using InVivoScope (Bioscan, Washington DC).

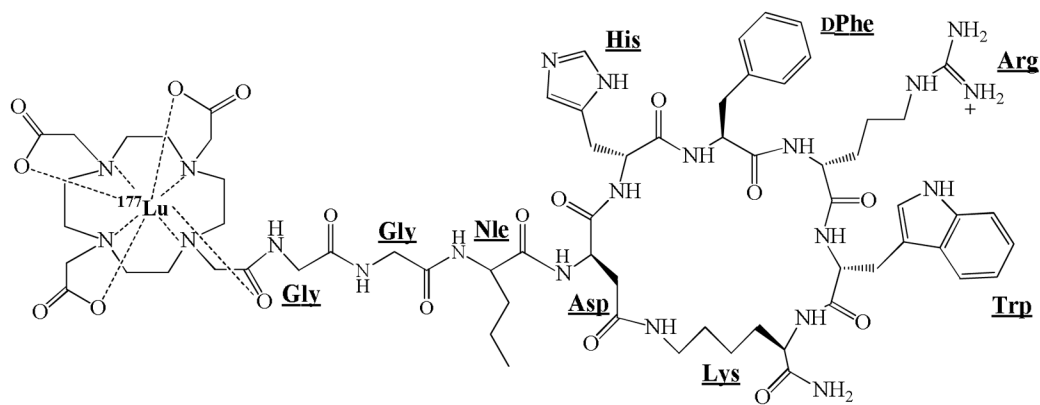


Figure 1.
Proposed schematic structure of ^{177}Lu -DOTA-GlyGlyNle-CycMSH_{hex}.

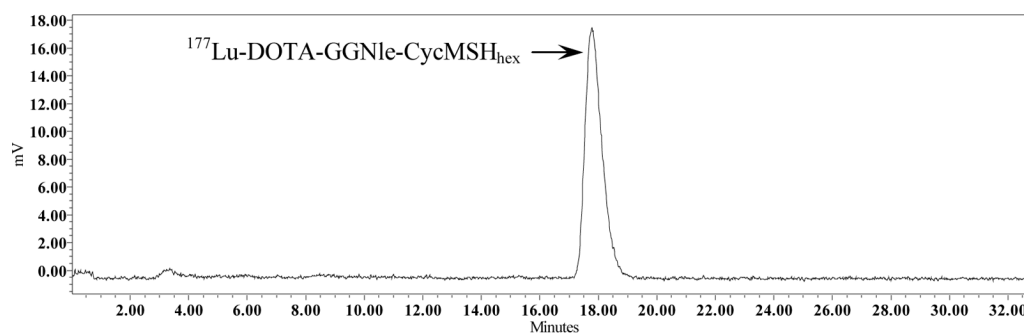


Figure 2. Serum stability of ^{177}Lu -DOTA-GlyGlyNle-CycMSH_{hex} after 24 h incubation at 37°C. The retention time of ^{177}Lu -DOTA-GlyGlyNle-CycMSH_{hex} was 17.8 min.

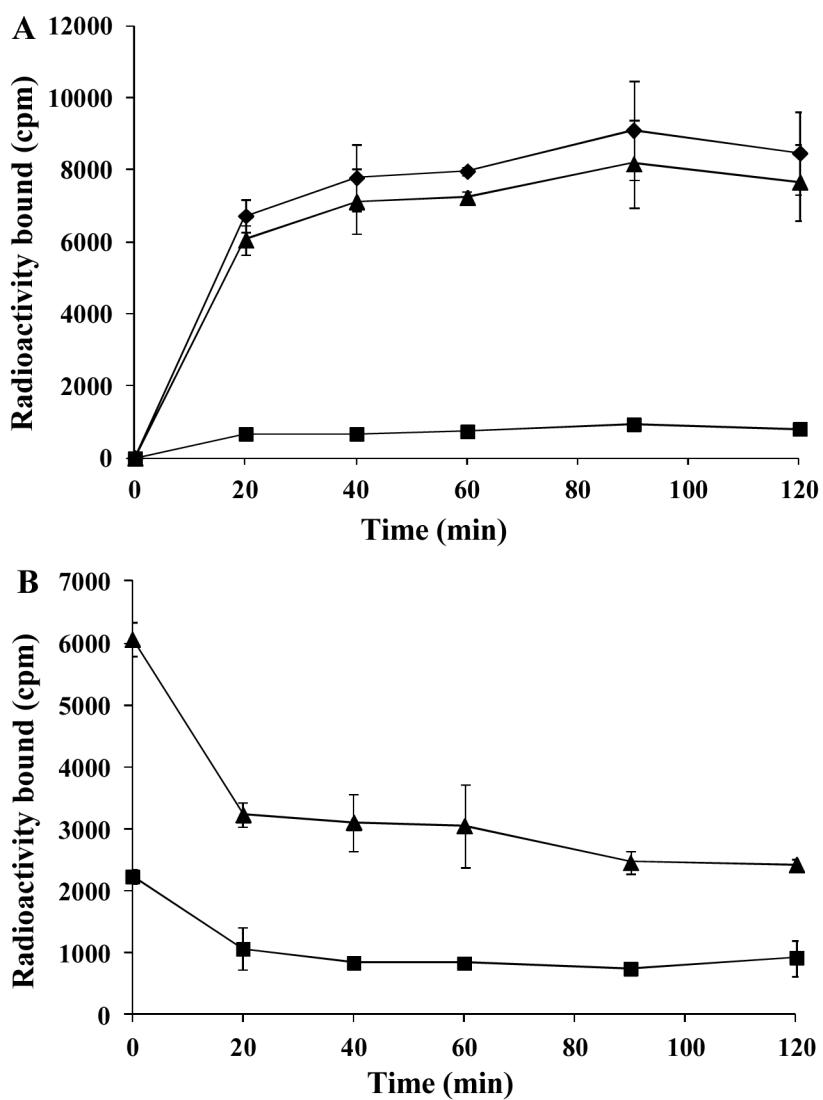


Figure 3. Cellular internalization (A) and efflux (B) of ^{177}Lu -DOTA-GlyGlyNle-CycMSH_{hex} in B16/F1 melanoma cells. Total bound radioactivity (◆), internalized radioactivity (▲) and cell membrane radioactivity (■) were presented as counts per minute (cpm).

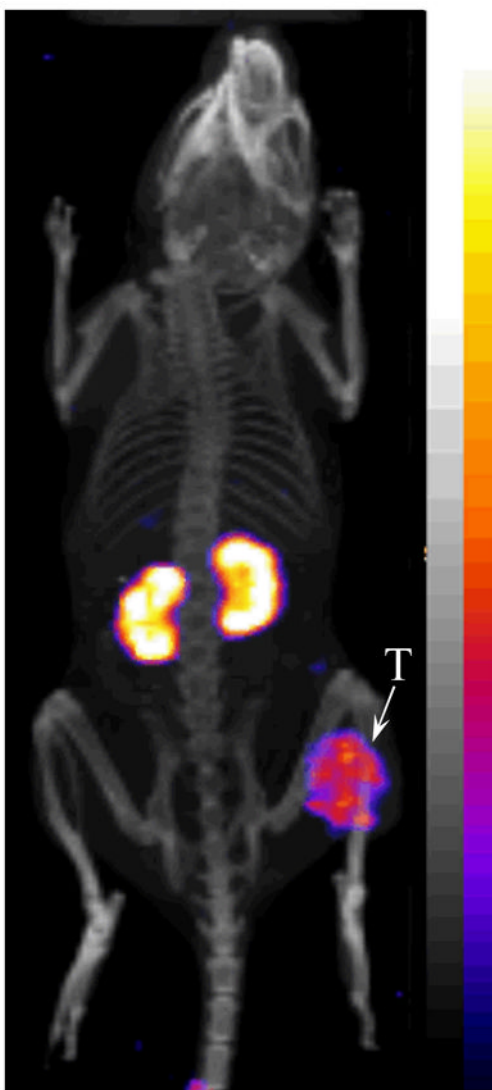


Figure 4. Representative whole-body SPECT/CT image of ^{177}Lu -DOTA-GlyGlyNle-CycMSH_{hex} in a B16/F1 melanoma-bearing C57 mouse at 2 h post-injection. The tumor lesions (T) were highlighted with an arrow on the image.

Table 1

Biodistribution of ^{177}Lu -DOTA-GlyGlyNle-CycMSh_{hex} in B16/F1 melanoma-bearing C57 mice. The data were presented as percent injected dose/gram or as percent injected dose (Mean \pm SD, n=5)

Tissues	0.5 h	2 h	4 h	24 h	2-h NDP blockade
Percent injected dose/gram (%ID/g)					
Tumor	20.25 \pm 4.59	21.63 \pm 6.27	15.78 \pm 1.45	8.24 \pm 1.51	0.81 \pm 0.20*
Brain	0.21 \pm 0.14	0.05 \pm 0.01	0.09 \pm 0.03	0.02 \pm 0.02	0.03 \pm 0.01
Blood	2.98 \pm 0.38	0.22 \pm 0.14	0.15 \pm 0.07	0.23 \pm 0.41	0.08 \pm 0.05
Heart	1.12 \pm 0.22	0.16 \pm 0.07	0.23 \pm 0.11	0.17 \pm 0.13	0.17 \pm 0.10
Lung	3.15 \pm 0.57	0.38 \pm 0.05	0.24 \pm 0.04	0.13 \pm 0.03	0.41 \pm 0.12
Liver	1.61 \pm 0.25	0.73 \pm 0.10	0.78 \pm 0.08	0.53 \pm 0.08	0.68 \pm 0.09
Spleen	1.21 \pm 0.19	0.27 \pm 0.14	0.32 \pm 0.11	0.24 \pm 0.10	0.25 \pm 0.11
Stomach	2.29 \pm 0.45	1.37 \pm 0.45	1.22 \pm 0.27	0.69 \pm 0.09	1.17 \pm 0.85
Kidneys	13.83 \pm 2.51	7.83 \pm 1.38	9.68 \pm 1.95	4.75 \pm 1.03	6.36 \pm 0.33
Muscle	1.05 \pm 0.34	0.17 \pm 0.09	0.05 \pm 0.02	0.14 \pm 0.05	0.24 \pm 0.12
Pancreas	0.88 \pm 0.32	0.20 \pm 0.05	0.24 \pm 0.09	0.20 \pm 0.11	0.21 \pm 0.06
Bone	0.95 \pm 0.33	0.34 \pm 0.10	0.64 \pm 0.33	0.18 \pm 0.24	0.57 \pm 0.32
Skin	3.28 \pm 0.50	0.48 \pm 0.06	0.50 \pm 0.09	0.33 \pm 0.06	0.29 \pm 0.20
Percent injected dose (%ID)					
Intestines	1.66 \pm 0.34	0.90 \pm 0.18	1.04 \pm 0.51	0.47 \pm 0.13	0.56 \pm 0.20
Urine	62.40 \pm 3.78	83.33 \pm 6.63	83.73 \pm 7.12	93.31 \pm 2.34	93.83 \pm 2.82
Uptake ratio of tumor/normal tissue					
Tumor/liver	12.58	29.63	20.23	15.55	1.19
Tumor/kidney	1.46	2.76	1.63	1.74	0.13
Tumor/lung	6.43	56.92	65.75	63.38	1.98
Tumor/muscle	19.29	127.24	315.6	58.86	3.38
Tumor/blood	6.80	98.32	105.2	35.83	10.13
Tumor/skin	6.18	45.06	31.56	24.97	2.79

* P<0.05 for determining significance of differences in tumor, kidney, liver, lung, muscle, skin and blood uptake between ^{177}Lu -DOTA-GlyGlyNle-CycMSh_{hex} with or without peptide blockade at 2 h post-injection.