Published in final edited form as:

Bioorg Med Chem Lett. 2017 October 15; 27(20): 4647–4651. doi:10.1016/j.bmcl.2017.09.004.

Evaluation of novel ^{111}In -labeled gonadotropin-releasing hormone peptides for human prostate cancer imaging

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Abstract

The purpose of this study was to evaluate the tumor targeting and imaging properties of novel ^{111}In -labeled gonadotropin-releasing hormone (GnRH) peptides for human prostate cancer. Three new 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA)-linker-D-Phe-(D-Lys⁶-GnRH) peptides with different hydrocarbon linkers were designed to evaluate their effects on GnRH receptor binding affinities. The Aoc (aminooctanoic acid) linker was better than βAla (beta alanine) and Aun (aminoundecanoic acid) linkers in retaining strong receptor binding affinity. DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) exhibited 6.6 ± 0.1 nM GnRH receptor binding affinity. ^{111}In -DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) exhibited fast tumor uptake and urinary clearance in DU145 human prostate cancer-xenografted nude mice. The DU145 tumor lesions could be clearly visualized by single photon emission computed tomography (SPECT)/CT using ^{111}In -DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) as an imaging probe, providing an insight into the design of new GnRH peptides for prostate cancer in the future.

Graphical Abstract

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Keywords

¹¹¹In-labeled gonadotropin-releasing hormone receptor; prostate cancer; single photon emission computed tomography

Prostate cancer is the most commonly diagnosed cancer in males (161,360 new cases) and the third leading cause of cancer-related death among men (26,730 fatalities) in the United States in 2017.¹ Approximately 30% of patients have metastases upon diagnosis^{2,3} and about 50% of patients eventually develop metastases.⁴ Survival times for patients with prostate cancer metastases are generally 2~3 years from the time when the metastases are diagnosed.⁵ Unfortunately, no curative treatment exists for metastatic prostate cancer. Early diagnosis is critical for appropriate treatment decisions and provides patients the best opportunities for cures or prolonged survivals.

We have been interested in developing radiolabeled gonadotropin-releasing hormone (GnRH) peptides to target GnRH receptors for prostate cancer imaging.⁶ Native GnRH peptide is a hypothalamic peptide with 10 amino acids (pGlu¹-His²-Trp³-Ser⁴-Tyr⁵-Gly⁶-Leu⁷-Arg⁸-Pro⁹-Gly¹⁰-NH₂). Both pGlu¹-His²-Trp³ and Arg⁸-Pro⁹-Gly¹⁰-NH₂ moieties are important for GnRH receptor binding, while the replacement of Gly⁶ with a D-amino acid decreases the metabolic clearance of the peptide and improve receptor binding affinity.^{7,8} Thus, the epsilon amino group of D-Lys⁶ in D-Lys⁶-GnRH peptide is commonly selected for conjugation of radionuclides.⁹⁻¹⁴ In our previous work, the radiometal chelator DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) was conjugated to D-Lys⁶-GnRH

via a lipophilic aminohexanoic acid (Ahx) linker to yield DOTA-Ahx-(D-Lys⁶-GnRH) peptide, which displayed 36.1 nM GnRH receptor binding affinity.⁶ Moreover, the DU145 human prostate cancer-xenografted tumor lesions were clearly visualized by single photon emission computed tomography (SPECT)/CT using ¹¹¹In-DOTA-Ahx-(D-Lys⁶-GnRH) as an imaging probe.⁶

The introduction of the lipophilic Ahx linker between the DOTA and (D-Lys⁶-GnRH) motif contributed to the success of ¹¹¹In-DOTA-Ahx-(D-Lys⁶-GnRH) as an imaging probe. Interestingly, we further found that the introduction of an aromatic amino acid of D-Phe between the Ahx and (D-Lys⁶-GnRH) motif substantially improved the binding affinity of DOTA-Ahx-D-Phe-(D-Lys⁶-GnRH) as compared to DOTA-Ahx-(D-Lys⁶-GnRH).¹⁵ This interesting finding further stimulated our interest regarding whether we could apply this strategy to improve the receptor binding affinity of other GnRH peptide with different hydrocarbon linkers. Therefore, we designed three new DOTA-conjugated D-Phe-D-Lys⁶-GnRH peptides with β Ala (3-aminopropanoic acid), Aoc (8-aminooctanoic acid) and Aun (11-aminoundecanoic acid) linkers between DOTA and D-Phe-D-Lys⁶-GnRH moiety. Then we determined their GnRH receptor binding affinities and selected the peptide with the strongest binding affinity for radiolabeling and in vivo evaluation.

DOTA- β Ala-D-Phe-(D-Lys⁶-GnRH), DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) and DOTA-Aun-D-Phe-(D-Lys⁶-GnRH) (Figure 1) were synthesized using 9-fluorenylmethyloxycarbonyl (Fmoc) chemistry and purified by reverse phase-high performance liquid chromatography (RP-HPLC). All peptides displayed greater than 95% purity after HPLC purification. The peptide identities were confirmed by electrospray ionization mass spectrometry. The found molecular weights of DOTA- β Ala-D-Phe-(D-Lys⁶-GnRH), DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) and DOTA-Aun-D-Phe-(D-Lys⁶-GnRH) matched their calculated molecular weights. The GnRH receptor binding affinities of DOTA- β Ala-D-Phe-(D-Lys⁶-GnRH), DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) and DOTA-Aun-D-Phe-(D-Lys⁶-GnRH) were determined using Millipore ChemoScreenTM human GnRH receptor membrane preparations. The IC₅₀ value of DOTA- β Ala-D-Phe-(D-Lys⁶-GnRH), DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) and DOTA-Aun-D-Phe-(D-Lys⁶-GnRH) was 13.5 $\pm$ 3.9, 6.6 $\pm$ 0.1 and 24.6 $\pm$ 2.5 nM, respectively (Figure 2). Interestingly, DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) displayed similar binding affinity as DOTA-Ahx-D-Phe-(D-Lys⁶-GnRH),¹⁵ whereas DOTA- β Ala-D-Phe-(D-Lys⁶-GnRH) and DOTA-Aun-D-Phe-(D-Lys⁶-GnRH) exhibited weaker binding affinity than that of DOTA-Ahx-D-Phe-(D-Lys⁶-GnRH). The receptor binding results suggested a profound linker effect in GnRH receptor binding. The Aoc and Ahx linkers were better than β Ala and Aun linkers by 2- to 3-fold in retaining the strong GnRH receptor binding in DOTA-linker-D-Phe-(D-Lys⁶-GnRH) peptides. Meanwhile, it was reported that the hydrocarbon linkers with 5- to 8-carbon between the DOTA and bombesin peptide resulted in 0.6–1.7 nM receptor binding affinities for DOTA-conjugated bombesin peptides. Either shorter or longer hydrocarbon linkers dramatically reduced the receptor binding affinity by 100-fold.¹⁶ Therefore, it is necessary to determine the specific linker effect among different peptides because the linker effect could be quite different among various peptides.

Since DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) showed the strongest GnRH receptor binding affinity, we radiolabeled DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) with ¹¹¹In for in further

evaluation. DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) was readily labeled with ¹¹¹In in 0.1 M 4-morpholineethanesulfonic acid (MES) buffer at pH 5.5 with greater than 95% radiolabeling yield. ¹¹¹In-DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) was completely separated from its excess non-labeled peptide by RP-HPLC. The retention time of DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) and ¹¹¹In-DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) was 13.6 and 16.6 min, respectively (Figure 3). The radiochemical purity of ¹¹¹In-DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) was greater than 98%. There was no detectable amount of DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) by UV detector after HPLC purification. The specific GnRH receptor binding of ¹¹¹In-DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) is shown in Figure 3. Incubation of DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) blocked 79% of the binding of ¹¹¹In-DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) ($p < 0.05$), indicating that the binding of ¹¹¹In-DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) was GnRH receptor-specific.

We determined the biodistribution properties of ¹¹¹In-DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) in DU145 human prostate cancer-xenografted nude mice to evaluate its ability to target GnRH receptor *in vivo*. The biodistribution results of ¹¹¹In-DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) are shown in Table 1. ¹¹¹In-DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) exhibited a rapid tumor uptake. The tumor uptake was 1.13 ± 0.36 and $0.88 \pm 0.2\%$ ID/g at 0.5 and 1 h post-injection. The tumor uptake decreased to 0.53 ± 0.11 and $0.32 \pm 0.03\%$ ID/g at 2 and 4 h post-injection. Co-injection of non-labeled DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) peptide significantly blocked the tumor uptake ($0.41 \pm 0.23\%$ ID/g at 1 h post-injection, $p < 0.05$), indicating that the tumor uptake of ¹¹¹In-DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) was GnRH receptor-mediated. The renal uptake was 8.01 ± 0.57 , 7.62 ± 0.83 , 7.83 ± 1.07 and $6.14 \pm 0.32\%$ ID/g at 0.5, 1, 2, and 4 h post-injection, respectively. The peptide blockade didn't decrease the renal uptake, suggesting that the renal uptake wasn't receptor-mediated. The whole-body clearance of ¹¹¹In-DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) was rapid, with approximately $82.15 \pm 1.54\%$ of the injected radioactivity cleared from the urinary system by 1 h post-injection. The urine metabolites are shown in Figure 4. Approximately 60% of ¹¹¹In-DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) remained intact, whereas 40% of ¹¹¹In-DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) was metabolized into other compounds with higher polarities. The imaging property of ¹¹¹In-DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) was determined in DU145 human prostate cancer-xenografted nude mice. The representative maximum intensity projection SPECT/CT image is presented in Figure 5. The DU145 xenografted tumor lesions were clearly visualized by SPECT/CT using ¹¹¹In-DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) as an imaging probe at 1 h post-injection.

We have demonstrated that the length of hydrocarbon linkers played a role in the receptor binding affinity of DOTA-linker-(D-Lys⁶-GnRH) peptides.¹⁴ For instance, DOTA-Aun-(D-Lys⁶-GnRH) exhibited stronger receptor binding affinity than DOTA-Ahx-(D-Lys⁶-GnRH) and DOTA-Aoc-(D-Lys⁶-GnRH) (22.8 vs. 26.6 and 36.1 nM).¹⁴ Interestingly, the introduction of aromatic D-Phe substantially improved the receptor binding affinities of DOTA-Ahx-D-Phe-(D-Lys⁶-GnRH)¹⁵ and DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) by 4.8- and 4-fold, respectively. However, the introduction of D-Phe didn't enhance the receptor binding affinity of DOTA-Aun-D-Phe-(D-Lys⁶-GnRH). Although the DU145 prostate tumor lesions were clearly visualized using ¹¹¹In-DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) as an imaging probe at 1 h post-injection, it is worthwhile to note that ¹¹¹In-DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH)

didn't exhibit higher tumor uptake as we anticipated. The urine analysis revealed that approximately 40% of ^{111}In -DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) was metabolized into other compounds with higher polarities at 1 h after injection. Therefore, it is possible that the rapid metabolism of ^{111}In -DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) *in vivo* didn't allow substantial percent of ^{111}In -DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) to reach GnRH receptors on tumor cells. Clearly, we need more work to better understand the metabolism of GnRH peptide and improve the tumor uptake. We have demonstrated that peptide cyclization via lactam bridge dramatically improved the tumor uptake of radiolabeled alpha-melanocyte stimulating hormone peptides.^{17–19} It would be interesting to investigate whether this strategy could improve the tumor uptake of radiolabeled GnRH peptides in the future.

The experimental details are presented in references and notes.^{20–26}

In conclusion, the introduction of D-Phe substantially enhanced the receptor binding affinity of DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH). ^{111}In -DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) exhibited rapid tumor uptake in DU145 human prostate cancer-xenografted nude mice. The DU145 tumor lesions could be clearly visualized by SPECT/CT using ^{111}In -DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) as an imaging probe, providing a new insight into the design of new radiolabeled GnRH peptides for prostate cancer in the future.

Acknowledgments

We appreciate Dr. Fabio Gallazzi for his technical assistance. This work was supported in part by the NIH grant R15CA173516 and University of Colorado Denver start-up fund.

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20. **Peptide synthesis:** Amino acids and resin were purchased from Advanced ChemTech Inc. (Louisville, KY) and Novabiochem (San Diego, CA). DOTA-tri-*tert*-butyl ester was purchased from Macrocyclics Inc. (Richardson, TX). Three new GnRH peptides were synthesized using 9-fluorenylmethyloxycarbonyl (Fmoc) chemistry according to our published procedure¹⁵ with modifications. Seventy micromoles of resin, 210 μ mol of each Fmoc-protected amino acid and 210 μ mol of (tBu)₃DOTA were used for the synthesis. The intermediate scaffold of pGlu-His(Trt)-Trp(Boc)-Ser(tBu)-Tyr(tBu)-D-Lys(Mtt)-Leu-Arg(Pbf)-Pro-Gly was synthesized on Rink amide resin by an Advanced ChemTech multiple-peptide synthesizer (Louisville, KY). The protecting group of Mtt in the scaffold was removed by 25% hexafluoroisopropanol (HFIP) in the presence of 10% *N,N*-diisopropylethylamine (DIPEA) for further conjugation of the moiety of D-Phe-BALA-DOTA(tBu)₃, D-Phe-Aoc-DOTA(tBu)₃, or D-Phe-Aun-DOTA(tBu)₃. After the conjugation, the protecting groups were totally removed by treating with a mixture of trifluoroacetic acid (TFA), thioanisole, phenol, water, ethanedithiol and triisopropylsilane (87.5:2.5:2.5:2.5:2.5) for 4 h at 25 °C. Each peptide was precipitated and washed with ice-cold ether four times, purified by reverse phase-high performance liquid chromatography (RP-HPLC) and characterized by LC-MS.
21. **In vitro GnRH receptor binding assay:** The GnRH receptor binding affinities (IC₅₀ values) of DOTA- β Ala-D-Phe-(D-Lys⁶-GnRH), DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) and DOTA-Aun-D-Phe-(D-Lys⁶-GnRH) were determined by *in vitro* competitive binding assay according to the published procedure.¹⁵ ¹²⁵I-[D-Trp⁶]-LH-RH {pGlu-His-Trp-Ser-Tyr-[¹²⁵I]-D-Trp-Leu-Arg-Pro-Gly-NH₂} was obtained from PerkinElmer Inc. (Boston, MA). The receptor binding assay was replicated in triplicate for each peptide. Briefly, 5 μ L of Millipore ChemoScreen™ human GnRH membrane preparations (Billerica, MA) were incubated at 25 °C for 3 h with approximately 30,000 counts per minute (cpm) of ¹²⁵I-[D-Trp⁶]-LH-RH in the presence of 10⁻¹² to 10⁻⁵ M of each peptide in 95 μ L of binding medium {50 mM *N*-(2-hydroxyethyl)-piperazine-*N'*-(2-ethanesulfonic acid), 5 mM MgCl₂, 1 mM CaCl₂, pH 7.4, 0.2% bovine serum albumin (BSA)}. After the incubation, 800 μ L of ice-cold washing buffer {50 mM *N*-(2-hydroxyethyl)-piperazine-*N'*-(2-ethanesulfonic acid), 500 mM NaCl, pH 7.4, 0.1% BSA} was added to each mixture. Each resulting mixture was filtered through a GF/C filter (Whatman, Clifton, NJ) pre-soaked in 1% of polyethylenimine. Each filter was rinsed with 1 mL of ice-cold washing buffer three times. The activities on the filters were measured in a Wallac 1480 automated gamma counter (PerkinElmer, Waltham, MA). The IC₅₀ value of each peptide was calculated using Prism software (GraphPad Software, La Jolla, CA).
22. **Peptide radiolabeling with ¹¹¹In:** ¹¹¹InCl₃ was purchased from MDS Nordion, Inc. (Vancouver, Canada) for radiolabeling. DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) showed the strongest GnRH receptor binding affinity among three peptides. Hence, we further evaluated DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH). ¹¹¹In-DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) was prepared in 0.5 M NH₄OAc at pH 5.2. Briefly, 120 μ L of 0.5 M NH₄OAc (pH 5.2), 10 μ L of DOTA-Aoc-D-Lys⁶-GnRH solution (1 mg/mL) and 50 μ L of ¹¹¹InCl₃ (37–74 MBq in 0.05 M HCl aqueous solution) were added into a reaction vial and incubated at 90 °C for 30 min. After the incubation, 10 μ L of 0.5% EDTA aqueous solution was added into the reaction vial to scavenge potential unbound ¹¹¹In³⁺ ions, and purified to single species by Waters RP-HPLC (Milford, MA, USA) on a Grace Vydac C-18 reverse phase analytic column (Deerfield, IL, USA) using a 20-min gradient of 18–26% acetonitrile in 20 mM HCl aqueous solution at a flow rate of 1 mL/min. The purified peptide was purged with N₂ gas at 25 °C for 20 mins to remove the acetonitrile. The pH of the final peptide solution was adjusted to 7.4 with 0.1 N NaOH and sterile normal saline for specific binding and animal studies.
23. **Specific human GnRH receptor binding of ¹¹¹In-DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH):** The specific human GnRH receptor binding of ¹¹¹In-DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) was determined using human GnRH receptor membrane preparations obtained from Millipore, Inc (Billerica, MA). Briefly, 50 μ L of Millipore ChemoScreen™ human GnRH receptor membrane preparations were incubated at 25 °C for 3 h with approximately 120,000 cpm of HPLC-purified ¹¹¹In-DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) in 50 μ L of binding medium with or without 1 μ M of DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) peptide blockade (n=3). After the incubation, each

membrane preparation was mixed with 800 μ L of ice-cold washing buffer first, and then filtered through a GF/C filter (Waterman, Clifton, NJ) pre-soaked in 1% polyethylenimine. Each filter was rinsed with 1 mL of ice-cold washing buffer three times and counted in a gamma counter. The experiment was replicated in triplicate.

24. **Biodistribution studies:** All the animal studies were conducted in compliance with Institutional Animal Care and Use Committee approval. DU145 human prostate cancer cells were obtained from American Type Culture Collection (Manassas, VA). The biodistribution of ^{111}In -DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) was determined in DU145 human prostate cancer-xenografted male athymic nude mice (Harlan, Indianapolis, IN). The nude mice were subcutaneously inoculated with 1×10^7 DU145 cells on the right flank of each mouse to generate DU145 xenografted tumors. The tumor weights reached approximately 0.3 g at 21 days post cell inoculation. Each tumor-bearing mouse was injected with 0.037 MBq of ^{111}In -DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) via the tail vein. Groups of 5 mice were sacrificed at 0.5, 1, 2 and 4 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 specificity of tumor uptake of ^{111}In -DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) was determined by co-injecting 10 μ g of unlabeled DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) at 1 h post-injection.
25. **Tumor imaging and urinary metabolites of ^{111}In -DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH):** To determine the tumor imaging property of ^{111}In -DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH), approximately 18.5 MBq of ^{111}In -DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) was injected into a DU 145 tumor-bearing nude mouse via the tail vein. The mouse was sacrificed at 1 h post-injection to collect the urine for analyzing the metabolites, as well as for small animal SPECT/CT (Nano-SPECT/CT, Bioscan) imaging. The CT scan 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 the In Vivo Scope (Bioscan, Washington, DC). The urine sample was directly centrifuged at 16,000g for 5 min before the HPLC analysis. Thereafter, the urine was injected into the HPLC using the gradient described above.
26. **Statistical methods:** Statistical analysis was performed using the Student *s t* test for unpaired data to determine the significance of differences in GnRH receptor binding, tumor, kidney and liver uptake between ^{111}In -DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) with or without 1 μ M of DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) peptide blockade. Difference at the 95% confidence level ($p < 0.05$) was considered significant.

1. DOTA- β Ala-D-Phe-(D-Lys⁶-GnRH)

2. DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH)

3. DOTA-Aun-D-Phe-(D-Lys⁶-GnRH)

Figure 1.

Schematic structure of DOTA-linker-D-Phe-(D-Lys⁶-GnRH) peptides.

Figure 2.

The competitive binding curves of the GnRH peptides. The IC_{50} values of DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH), DOTA- β -Ala-D-Phe-(D-Lys⁶-GnRH) and DOTA-Aun-D-Phe-(D-Lys⁶-GnRH) were 6.6 ± 0.1 , 13.5 ± 3.9 and 24.6 ± 2.5 nM, respectively.

Figure 3.

Radioactive HPLC profile of ^{111}In -DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) (A) and UV HPLC profile of DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) (B). The retention times of ^{111}In -DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) and DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) were 16.6 and 13.6 min, respectively. Binding of ^{111}In -DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) on human GnRH receptor membrane preparations with (white) or without (black) the presence of 1 μM of DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH).

Figure 4.

Radioactive HPLC profile of the urine sample of a DU145 human prostate cancer-xenografted nude mouse at 1 h post-injection of ^{111}In -DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH). The arrow indicates the retention time of the original ^{111}In -DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH) prior to the tail vein injection.

Figure 5. Representative maximum intensity projection SPECT/CT image of DU145 human prostate cancer-xenografted tumor at 1 h post-injection of 18.5 MBq of ^{111}In -DOTA-Aoc-D-Phe-(D-Lys⁶-GnRH). Flank tumor lesions (T) are highlighted with an arrow on the image.

Biodistribution of ^{111}In -DOTA-Aoc-D-Phe-(D-Lys 6 -GnRH) in DU145 human prostate cancer-xenografted nude mice. The data was presented as percent injected dose/gram or as percent injected dose (mean $\pm$ SD, n = 5)

Table 1

Tissue	0.5 h	1 h	1 h peptide blockade	2 h	4 h
	Percent injected dose/gram (%ID/g)				
Tumor	1.13 $\pm$ 0.36	0.88 $\pm$ 0.20	0.41 $\pm$ 0.23 *	0.53 $\pm$ 0.11	0.32 $\pm$ 0.03
Brain	0.09 $\pm$ 0.04	0.11 $\pm$ 0.05	0.09 $\pm$ 0.07	0.04 $\pm$ 0.01	0.03 $\pm$ 0.03
Blood	1.47 $\pm$ 0.42	0.58 $\pm$ 0.21	0.46 $\pm$ 0.09	0.15 $\pm$ 0.11	0.07 $\pm$ 0.05
Heart	0.65 $\pm$ 0.11	0.20 $\pm$ 0.11	0.17 $\pm$ 0.06	0.11 $\pm$ 0.06	0.12 $\pm$ 0.09
Lung	1.61 $\pm$ 0.24	0.58 $\pm$ 0.10	0.72 $\pm$ 0.16	0.21 $\pm$ 0.04	0.12 $\pm$ 0.08
Liver	1.56 $\pm$ 0.22	0.68 $\pm$ 0.11	0.86 $\pm$ 0.22	0.50 $\pm$ 0.07	0.31 $\pm$ 0.12
Spleen	0.67 $\pm$ 0.16	0.41 $\pm$ 0.18	0.33 $\pm$ 0.14	0.33 $\pm$ 0.24	0.16 $\pm$ 0.08
Stomach	1.11 $\pm$ 0.66	0.61 $\pm$ 0.28	0.42 $\pm$ 0.30	0.26 $\pm$ 0.15	0.35 $\pm$ 0.10
Kidneys	8.01 $\pm$ 0.57	7.62 $\pm$ 0.83	9.63 $\pm$ 1.47	7.83 $\pm$ 1.07	6.14 $\pm$ 0.32
Muscle	0.53 $\pm$ 0.10	0.27 $\pm$ 0.08	0.15 $\pm$ 0.04	0.11 $\pm$ 0.03	0.15 $\pm$ 0.07
Pancreas	0.74 $\pm$ 0.26	0.32 $\pm$ 0.07	0.24 $\pm$ 0.06	0.16 $\pm$ 0.08	0.08 $\pm$ 0.02
Bone	1.24 $\pm$ 0.24	0.88 $\pm$ 0.42	0.36 $\pm$ 0.13	0.34 $\pm$ 0.01	0.09 $\pm$ 0.03
	Percent injected dose (%ID)				
Intestines	1.09 $\pm$ 0.19	0.87 $\pm$ 0.18	1.10 $\pm$ 0.64	2.57 $\pm$ 0.34	2.37 $\pm$ 0.15
Urine	59.90 $\pm$ 5.96	82.15 $\pm$ 1.54	83.54 $\pm$ 4.25	82.15 $\pm$ 7.84	90.54 $\pm$ 2.53
	Uptake ratio of tumor/normal tissue				
Tumor/Blood	0.77	1.52	0.89	3.53	4.57
Tumor/Muscle	2.13	3.26	2.73	4.82	2.13

* $p < 0.05$, significance of difference in tumor uptake between ^{111}In -DOTA-Aoc-D-Phe-(D-Lys 6 -GnRH) with or without peptide blockade at 1 h post-injection.