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Influences of hydrocarbon linkers on the receptor binding affinities of gonadotropin-releasing hormone peptides

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ABSTRACT

Three new DOTA-conjugated GnRH peptides with various hydrocarbon linkers were synthesized to evaluate the influences of the linkers on their receptor binding affinities. The hydrocarbon linker displayed a profound impact on the receptor binding affinities of DOTA-conjugated GnRH peptides. The Aun linker was better than Gaba, Ahx and Aoc linkers in retaining strong receptor binding affinity of the GnRH peptide. DOTA-Aun-(D-Lys^6-GnRH) displayed 22.8 nM GnRH receptor binding affinity. ^{111}In-DOTA-Aun-(D-Lys^6-GnRH) exhibited fast tumor uptake and urinary clearance in MDA-MB-231 human breast cancer-xenografted nude mice. The cellular and biological results provided an insight into the design of new GnRH peptides in the future.

Breast cancer was the most commonly diagnosed cancer in females (232,340 new cases) and the second leading cause of cancer-related death among women (39,620 fatalities) in the United States in 2013.[1] Unfortunately, no curative treatment exists for metastatic breast cancer. Early diagnosis followed by a prompt surgical removal provides patients the best opportunities for cures or prolonged survivals. Gonadotropin-releasing hormone (GnRH) receptor is a distinct molecular target due to its over-expression on breast cancer cells,[2–6] as well as on breast cancer specimens.[5,6] Over the past few years, we have been interested in developing radiolabeled GnRH peptides to target the GnRH receptors for cancer imaging.[7,8] Native GnRH peptide is a peptide with 10 amino acids (pGlu^1-His^2-Trp^3-Ser^4-Tyr^5-Gly^6-Leu^7-Arg^8-Pro^9-Gly^{10}-NH$_2$). In our previous report,[7] we replaced the Gly^6 with D-Lys^6 and conjugated the radiometal chelator DOTA {1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid} to the epsilon or alpha amino group of D-Lys^6 via an aminohexanoic acid (Ahx) hydrocarbon linker to yield new DOTA-conjugated GnRH peptides. Interestingly, we found that the epsilon amino group was a better position than the alpha amino group of D-Lys^6 for DOTA coupling in terms of retaining the nanomolar GnRH receptor binding.[7]

In this study, we managed to further improve the receptor binding affinities of DOTA-conjugated GnRH peptides building upon the success of DOTA-Ahx-(D-Lys^6-GnRH1). Because both pGlu^1-His^2-Trp^3 and Arg^8-Pro^9-Gly^{10} motifs were critical for receptor binding,[9] we tried to use hydrocarbon linkers with different lengths to improved the receptor binding affinities of the peptides. We hypothesized that a longer hydrocarbon linker would be better than a shorter hydrocarbon linker in enhancing the GnRH receptor binding affinity of the peptide. To examine the hypothesis, we substituted the Ahx linker with Gaba (gamma-aminobutyric acid), Aoc (aminooctanoic acid) and Aun (aminoundecanoic acid) linkers with different lengths to yield three new DOTA-conjugated GnRH peptides in this study. Specifically, the DOTA was conjugated to the epsilon amino group of D-Lys^6 via Gaba, Aoc and Aun linkers to generate DOTA-Gaba-(D-Lys^6-GnRH), DOTA-Aoc-(D-Lys^6-GnRH) and DOTA-Aun-(D-Lys^6-GnRH) peptides (Fig. 1). The peptides were synthesized on Rink amide resin using 9-fluorenylmethyloxycarbonyl (Fmoc) chemistry according to our published procedure.[7] All three peptides were purified by reverse phase-high performance liquid chromatography (RP-HPLC) and characterized by electrospray ionization mass spectrometry. We successfully synthesized DOTA-Gaba-(D-Lys^6-GnRH), DOTA-Aoc-(D-Lys^6-GnRH) and DOTA-Aun-(D-Lys^6-GnRH) with 30% overall yields. The measured molecular weights of DOTA-Gaba-(D-Lys^6-GnRH) DOTA-Aoc-(D-Lys^6-GnRH) and DOTA-Aun-(D-Lys^6-GnRH) were 1723.4, 1780.6 and 1822.5, respectively. The peptides displayed greater than 90% purities after the HPLC purification.

The receptor binding affinities of DOTA-Gaba-(D-Lys^6-GnRH), DOTA-Aoc-(D-Lys^6-GnRH) and DOTA-Aun-(D-Lys^6-GnRH) were determined in human GnRH receptor membrane preparations

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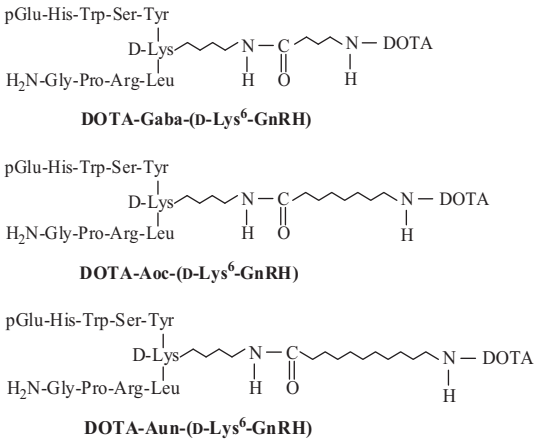

Figure 1. Schematic structures of DOTA-linker-(D-Lys^6-GnRH) peptides.

obtained from Millipore, Inc (Billerica, MA) according to our published procedure.[7] The receptor binding results are presented in Figure 2. Interestingly, the length of hydrocarbon linker displayed a profound effect on the GnRH receptor binding affinity of the peptide. As compared to DOTA-Ahx-(D-Lys^6-GnRH1) with 36.1 nM GnRH receptor binding affinity, the replacement of Ahx linker with the shorter Gaba linker dramatically decreased the GnRH receptor binding affinity of DOTA-Gaba-(D-Lys^6-GnRH) by 3.7-fold to 134.5 nM. On the other hand, the substitution of Ahx linker with the longer Aoc and Aun linkers increased the GnRH receptor binding affinities of DOTA-Aoc-(D-Lys^6-GnRH) and DOTA-Aun-(D-Lys^6-GnRH). Both DOTA-Aoc-(D-Lys^6-GnRH) and DOTA-Aun-(D-Lys^6-GnRH) retained low nanomolar GnRH receptor binding affinities (26.6 and 22.8 nM, respectively). Because DOTA-Aun-(D-Lys^6-GnRH) exhibited the strongest receptor binding affinity among the three new peptides, we further radiolabeled DOTA-Aun-(D-Lys^6-GnRH) peptide with ^{111}In and determined its specific GnRH receptor binding.

DOTA-Aun-(D-Lys^6-GnRH) was radiolabeled with ^{111}In in 0.5 M NH_4OAc buffer at pH 4.5. ^{111}In-DOTA-Aun-(D-Lys^6-GnRH) was readily prepared with greater than 95% radiolabeling yield and was completely separated from its excess non-radiolabeled peptide by RP-HPLC. The retention time of ^{111}In-DOTA-Aun-(D-Lys^6-GnRH) was 19.2 min. ^{111}In-DOTA-Aun-(D-Lys^6-GnRH) was stable in mouse serum at 37 °C for 24 h (Fig. 3) that warranted its further evaluation. The specific GnRH receptor binding of ^{111}In-DOTA-Aun-(D-Lys^6-GnRH) was determined on human GnRH receptor preparations. Approximately 85% of the binding of ^{111}In-DOTA-Aun-(D-Lys^6-GnRH) was competed off by 1 μM of DOTA-Aun-(D-Lys^6-GnRH) peptide (Fig. 3), demonstrating that the binding of ^{111}In-DOTA-Aun-(D-Lys^6-GnRH) was GnRH receptor-specific.

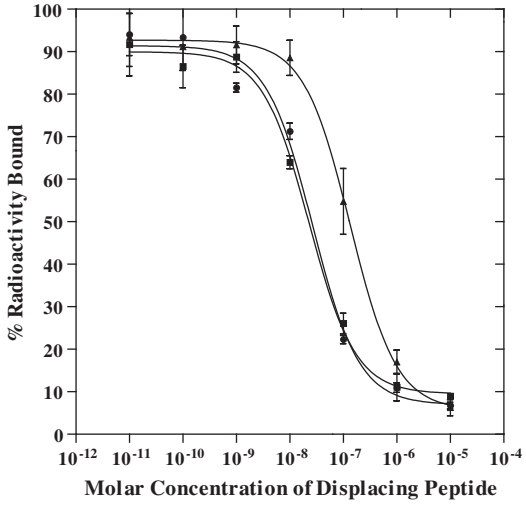

Figure 2. The competitive binding curves of the GnRH peptides. The IC_{50} values of DOTA-Gaba-(D-Lys^6-GnRH) (▲), DOTA-Aoc-(D-Lys^6-GnRH) (●) and DOTA-Aun-(D-Lys^6-GnRH) (■) were 134.5, 26.6 and 22.8 nM, respectively.

The GnRH receptor expression was positively stained in MDA-MB-231 human breast cancer-xenografted tumors in our previous report.[7] Thus, we determined the tumor targeting and pharmacokinetic properties of ^{111}In-DOTA-Aun-(D-Lys^6-GnRH) in MDA-MB-231 human breast cancer-xenografted nude mice. The biodistribution results of ^{111}In-DOTA-Aun-(D-Lys^6-GnRH) are presented in Table 1. ^{111}In-DOTA-Aun-(D-Lys^6-GnRH) exhibited rapid tumor uptake. The tumor uptake was 1.77 ± 0.22 and 1.10 ± 0.25% ID/g at 0.5 and 1 h post-injection. The tumor uptake decreased to 0.68 ± 0.08 and 0.29 ± 0.12% ID/g at 2 and 4 h post-injection. As compared to DOTA-Ahx-(D-Lys^6-GnRH), the enhancement in receptor binding affinity of DOTA-Aun-(D-Lys^6-GnRH) also resulted in the improvement in tumor uptake in MDA-MB-231 human breast cancer-xenografted nude mice. The tumor uptake of ^{111}In-DOTA-Aun-(D-Lys^6-GnRH) was 2.3 and 1.9 times the tumor uptake of ^{111}In-DOTA-Ahx-(D-Lys^6-GnRH) at 2 and 4 h post-injection, respectively. Meanwhile, it is important to note that the tumor/blood and tumor/muscle ratios of ^{111}In-DOTA-Aun-(D-Lys^6-GnRH) were higher than these of ^{111}In-DOTA-Ahx-(D-Lys^6-GnRH) at 4 h post-injection. The tumor/blood ratio of ^{111}In-DOTA-Aun-(D-Lys^6-GnRH) was 1.5 times the tumor/blood ratio of ^{111}In-DOTA-Ahx-(D-Lys^6-GnRH), whereas the tumor/muscle ratio of ^{111}In-DOTA-Aun-(D-Lys^6-GnRH) was 7.7 times the tumor/muscle ratio of ^{111}In-DOTA-Ahx-(D-Lys^6-GnRH) at 4 h post-injection. ^{111}In-DOTA-Aun-(D-Lys^6-GnRH) displayed rapid whole-body clearance, with approximately 88% of the injected radioactivity cleared through the urinary system by 2 h post-injection. The kidneys were the normal organs with the highest uptakes after 2 h post-injection. The renal uptake was 14.20 ± 3.08, 12.71 ± 1.42, 8.12 ± 0.69 and 2.62 ± 1.24% ID/g at 0.5, 1, 2 and 4 h post-injection, respectively.

Hydrocarbon linker also displayed a profound impact on the receptor binding affinities of DOTA-conjugated bombesin peptides.[10] It was reported that the DOTA-conjugated bombesin peptides with the linkers ranging from 5-carbon (Ava) to 8-carbon (Aoc) exhibited 0.6–1.7 nM receptor binding affinities. Either shorter or longer hydrocarbon linkers dramatically reduced the receptor binding affinity by 100-fold.[10] In this study, DOTA-Aun-(D-Lys^6-GnRH) displayed the strongest receptor binding affinity among four DOTA-conjugated GnRH peptides with Gaba, Ahx, Aoc and Aun linkers. In other words, the Aun linker was better than Gaba, Ahx and Aoc linkers in terms of retaining strong receptor binding affinity of the GnRH peptide. Despite that the hydrocarbon linker displayed a profound impact on the receptor binding affinities of both bombesin and GnRH peptides, the optimal linker length for strongest receptor binding affinity was different between the bombesin and GnRH peptides. Thus, it is important and necessary to determine the optimal linker length for strong receptor binding when designing new receptor-targeting peptides.

The experimental details are presented in References and notes.[11–13]

In conclusion, the hydrocarbon linker displayed a profound impact on the receptor binding affinities of DOTA-conjugated GnRH peptides. The Aun linker was better than Gaba, Ahx and Aoc linkers in terms of retaining strong receptor binding affinity of the GnRH peptide. ^{111}In-DOTA-Aun-(D-Lys^6-GnRH) exhibited fast tumor uptake and urinary clearance in MDA-MB-231 human breast cancer-xenografted nude mice. The cellular and biological results