In vitro Evaluation of a Bombesin Antagonistic Analogue Conjugated with DOTA-Ala(SO_3H)-Aminooctanoyl for Targeting of the Gastrin-releasing Peptide Receptor

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1. Introduction

The Gastrin-releasing peptide receptor (GRPR) has been shown to be overexpressed on many human tumors, including prostate, colon, gastric, breast, pancreatic, and small cell lung cancers [1]. As Bombesin (BBS) binds with high affinity to GRPR, BBS derivatives have been labeled with various radionuclides such as ^{99m}Tc, ^{111}In, ^{90}Y, ^{64}Cu, ^{177}Lu, ^{68}Ga, or ^{18}F and have proved to be successful candidates for peptide receptor radiotherapy (PRRT) [2,3]. In this study, we employed Ala(SO_3H)-Aminooctanoyl as a linker of BBS antagonistic peptide sequence, Gln-Trp-Ala-Val-N methyl Gly-His-Statine-Leu-NH_2, with DOTA to prepare radiolabeled candidates for GRPR targeting. A DOTA-conjugated BBS antagonistic analogue was synthesized and radiolabeled with ^{177}Lu, and *in vitro* characteristics on GRPR-overexpressing human prostate tumor cells were evaluated.

2. Methods and Results

2.1 Preparation of chelator conjugated peptide

Since a ^{177}Lu-labeled BBS agonist showed several side effects including abdominal cramps, diarrhea, and nausea in clinical phase I, a lot of reports have hypothesized that these side effects may be absent when using BBS antagonists [4]. Therefore, we set out to synthesize a BBS antagonist for labeling with ^{177}Lu, in which the final peptide sequence was DOTA-Ala(SO_3H)-Aminooctanoyl-Gln-Trp-Ala-Val-N methyl Gly-His-Statine-Leu-NH_2 (DOTA-sBBNA).

The peptide was prepared using a solid-phase synthetic method, and the final structural formula was as shown in Fig. 1. A purity of the peptide was over 98%, and the final MS data of the peptide were equal to the calculated value of the proposed formula (mw = 1658).

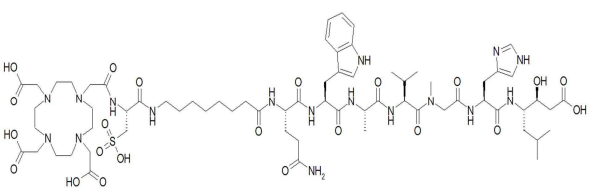


Fig. 1 The structural formula of DOTA-sBBNA.

2.2 Radio-labeling of the peptide with ^{177}Lu

^{177}Lu-DOTA-sBBNA was prepared at a high yield (> 98 %) by adding $^{177}LuCl_3$ to an aqueous solution (pH 5.5 sodium acetate) of the peptides at 90 ℃ for 30 min. As shown in Fig. 2, the HPLC chromatogram of ^{177}Lu-DOTA-sBBNA showed a retention time of 11.3 min.

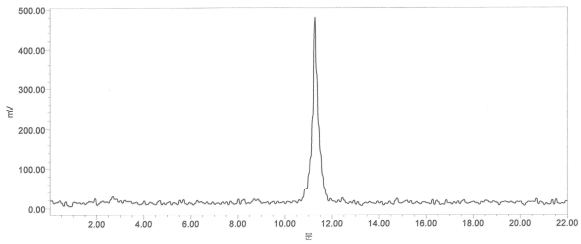
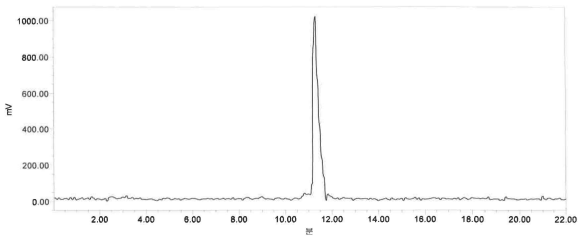


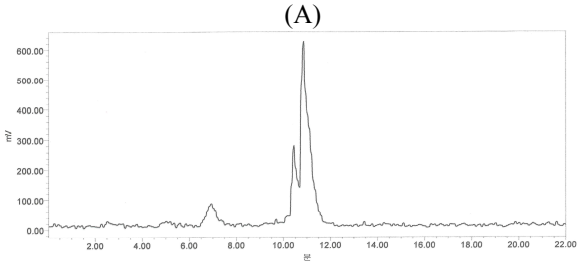
Fig. 2 Typical profiles of ^{177}Lu-DOTA-sBBNA determined by HPLC analysis using a C-18 column.

2.3 Stability of ^{177}Lu-labeled peptide

^{177}Lu-labeled peptides were stable in human serum at 37 ℃ for 2 days. The *in vivo* stability of ^{177}Lu-DOTA-sBBNA was also analyzed in murine urine by the administration of 7.4 MBq ^{177}Lu-DOTA-sBBNA into Balb/c nude mice. Similar to another radiolabeled-BBS antagonist, ^{18}F-BAY 86-4367, 3 radio-metabolites could be detected in the urine [3].



(A)



(B)

Fig. 3 *In vitro* stability of ^{177}Lu-DOTA-sBBNA in human serum (A) and i*n vivo* metabolism of radiolabeled peptide in murine urine (B).

2.4 Binding Affinity on GRPR

The *in vitro* GRPR binding affinities and specificities of DOTA-sBBNA were assessed using a competitive displacement assay with ^{125}I-[Tyr^4]-BBN as the radioligand on GRPR-over-expressing PC-3 prostate carcinoma cells. Compared with ^{18}F-BAY 86-4367 (IC_{50} = 0.94 nM), DOTA-sBBNA still retained reasonable affinity to GRPR (IC_{50} = 6.76 nM), as shown in Fig. 4. Additionally, a receptor saturation assay revealed that Kd of ^{177}Lu-DOTA-sBBNA was 1.88 nM which also indicated a highly nanomolar binding affinity. These results were encouraging to apply the peptide as a targeting modality for a GRPR-over-expressing PC-3 tumor.

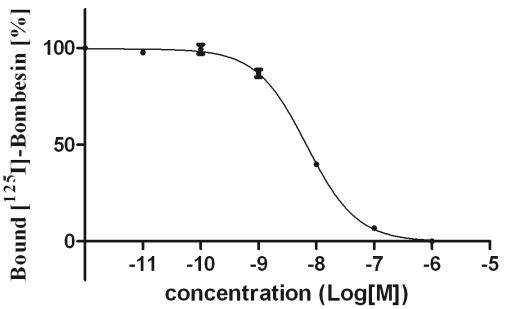


Fig. 4 Competitive binding of ^{125}I-[Tyr^4]-Bombesin on PC-3 cells by a treatment of DOTA-sBBNA.

2.5 Internalization characteristics

Internalization was performed in 6 well plates as described by Hanwen et al [5]. As shown in Fig. 5, ^{177}Lu-labeled radiopeptide showed a fast cell uptake, which reached a plateau within 1 hour of incubation at 37℃ in PC-3 cells. The peptide was slowly internalized, and < 25% of the added peptide was internalized for 4 hrs. Another BBS antagonistic analogue such as ^{64}Cu-(NO2A-8 Aoc-D-Phe^6-BBN(6-13)NHEt)] also reached a plateau of between 60 and 120 min, however, a much higher amount of ^{177}Lu-DOTA-sBBNA was internalized [6].

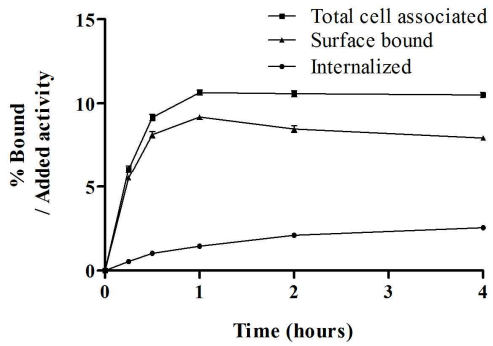


Fig. 5 Internalization rate of ^{177}Lu-DOTA-sBBNA into PC-3 cells.

3. Conclusions

In conclusion, a novel BBS antagonistic analogue, ^{177}Lu-DOTA-sBBNA, is a promising candidate for the targeting of GRPR-over-expressing tumors. Further investigations to evaluate its *in vivo* characteristics and therapeutic efficacy are needed.

Acknowledgement

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