Synthesis, Radiolabeling and Gastrin Releasing Peptide Receptor Binding Affinity of a Novel Bombesin Antagonist-Based Peptide, DOTA-Ala(SO_3H)-Aminooctanoyl-Gln-Trp-Ala-Val-N methyl Gly-His-Statine-Leu-NH_2

Jae Cheong Lim*, Sang Mu Choi, Eun Ha Cho and Jin Joo Kim

Radioisotope Research Division, Department of Research Reactor Utilization, Korea Atomic Energy Research Institute, Daejeon 305-353, Korea

Abstract - Bombesin receptors are overexpressed in many kinds of human tumors. In particular, the gastrin releasing peptide receptor (GRPR) which is also called bombesin receptor subtype 2, has been identified in prostate cancer. In the present study, we developed a bombesin antagonist-based ^{177}Lu-labeled peptide, ^{177}Lu-DOTA-Ala(SO_3H)-Aminooctanoyl-Gln-Trp-Ala-Val-N methyl Gly-His-Statine-Leu-NH_2 (DOTA-sBBNA). DOTA-sBBNA was prepared using a solid phase synthesis method. It was labeled with ^{177}Lu by a high radiolabeling yield (>98%), and its Log *P* value was −2.05. The radiolabeled peptide was highly stable in serum incubation at 37°C for 48 hr. A competitive displacement of ^{125}I-[Tyr^4]-Bombesin on the PC-3 human prostate carcinoma cells revealed that the IC_{50} value of the peptide was 6.76 nM indicating a highly nanomolar binding affinity for GRPR. These results suggest that ^{177}Lu-DOTA-sBBNA can be a potential candidate for targeting prostate cancer, and further studies to evaluate its biological characteristics are needed.

Key words : ^{177}Lu, Bombesin, Antagonist, Gatrin releasing peptide receptor, Tumor targeting

INTRODUCTION

Peptide receptors are used as targets for the imaging and targeted radionuclide therapy for cancer. The receptors of the Bombesin (BBS) family are very suitable as a target because they are overexpressed in a variety of human cancers. In particular, the gastrin releasing peptide receptor (GRPR) has been identified in prostate and breast cancers, gastrointestinal stromal tumors, and peritumoral vessels in ovarian cancer (Gugger and Reubi 1999; Markwalder and Reubi 1999; Reubi *et al.* 2004; Fleischmann *et al.* 2007; Abiraj *et al.* 2011). A lot of bombesin (BBS) analogues have been labeled with various radionuclides such as ^{99m}Tc, ^{111}In, ^{90}Y, ^{64}Cu, ^{177}Lu, ^{68}Ga, or ^{18}F for a diagnosis and treatment of GRPR positive prostate tumor (Smith *et al.* 2003; Okarvi 2004; Smith *et al.* 2005; Mu *et al.* 2010), however, none of them seem to have provided a breakthrough in clinical applications thus far.

Recently, ^{177}Lu-AMBA, which is ^{177}Lu-labeled DOTA-conjugated BBS7-14, was studied in clinical phase I and showed several side effects including abdominal cramps, diarrhea, and nausea (Bodei and Nunn 2007). Additionally, agonists of the BBS family showed their mitogenic properties (Casanueva *et al.* 1996). Thus, a lot of reports have hypothesized that these side effects may be absent when using BBS antagonists (Abd-Elgaliel *et al.* 2008; Cescato *et al.* 2008; Mansi *et al.* 2009; Abiraj *et al.* 2011; Mansi *et al.* 2011), and the antagonistic properties of the peptide sequence, Gln-Trp-Ala-Val-N methyl Gly-His-Statine-Leu-NH_2 was reported

* Corresponding author: Jae Cheong Lim, Tel. +82-42-868-8344, Fax. +82-42-868-8448, E-mail. limjc@kaeri.re.kr

(Honer *et al.* 2011).

In general, bifunctional chelating agents (BFCA) are used for the preparation of radiolabeled compounds, in order to introduce radionuclides to the targeting molecules without any distortion of their structure and not reduce their binding affinity and stability. Among them, the DOTA is able to strongly chelate many radionuclides such as ^{68}Ga, ^{111}In, ^{149}Pm, ^{212}Pb, ^{90}Y, and ^{177}Lu (Ruegg 1990; Depalatis 1995; Pippin 1995; Kukis 1998; Kwekkeboom 1999). In particular, ^{177}Lu emits medium and lower-energy β-rays (497 keV), and thus ^{177}Lu is considered a suitable radionuclide for treating small tumors or metastatic deposits. In addition, ^{177}Lu emits γ-rays (113 and 208 keV, 6% and 11%), which allows scintigraphic imaging and dosimetry (Miao *et al.* 2005).

The present study describes the synthesis, radiolabeling, and *in vitro* competitive binding of a novel BBN-related peptide antagonist, ^{177}Lu-DOTA-Ala(SO_3H)-Aminooctanoyl-Gln-Trp-Ala-Val-N methyl Gly-His-Statine-Leu-NH_2 (^{177}Lu-DOTA-sBBNA) to evaluate its possibility for the targeting of GRPR over-expressing tumors.

MATERIALS AND METHODS

1. Materials

All chemicals were of analytical grade purchased from a chemical company, and used without further purification. Automated solid-phase synthesis was accomplished through the use of a Multiple Biomolecular Synthesizer (Peptron, Daejeon, Republic of Korea). Analytical and preparative RP-HPLC was performed on a SHIMAZU prominence HPLC using a Shiseido capcell pak ^{18}C column. A wavelength of 220 nm was used for UV detection for analytical RP-HPLC. The LC/MS was performed using an HP 1100 series. ^{177}Lu was purchased from Perkin-Elmer (Massachusetts, USA) and the radioactivity was measured using an ionizing chamber (Atomlab 200, Bio-dex, New York, USA). The radiolabeling yield and radiochemical purity (RCP) were determined using a gamma detector-equipped HPLC analyzer (Waters, Milford, USA).

2. Preparation of chelator conjugated peptides

The peptide was prepared through the use of an automated Multiple Biomolecular Synthesizer (Peptron, Daejeon, Republic of Korea). The DOTA-sBBNA was synthesized by applying a standard Fmoc (fluorenylmethyloxycarbonyl) strategy as detailed in Fig. 1(A). Briefly, Fmoc-Leu-OH conjugated 4-methylbenzhydrylamine (MBHA) resin was used as an anchor polymeric support for a solid phase synthesis. After removing the Fmoc protecting group from resin-bounded Fmoc-Leu-OH under a standard cleavage condition (20% Piperidine in N,N-Dimethylformamide), the linear sequence peptide was prepared through the sequential coupling of Fmoc-Statine-OH, Fmoc-His(Trt)-OH, Fmoc-N methyl Gly-OH, Fmoc-Val-OH, Fmoc-Ala-OH, Fmoc-Trp(tBoc)-OH, Fmoc-Gln(Trt)-OH, Fmoc-8 aminooctanoic acid, and Fmoc-Ala(SO_3H). DOTA($OtBu)_3$ was introduced into the peptide by applying HBTU, HOBt, DIPEA and DMF as an activating reagent to ensure efficient coupling. The resulting peptide was cleaved from the polymeric support by treatment with a mixture solvent of 90% TFA containing 2.5% triisopropylsilane (TIS), 2.5% ethanedithiol (EDT), 2.5% thioanisole, and 2.5% deionized water (TFA : TIS : EDT : Thioanisole : H_2O=90 : 2.5 : 2.5 : 2.5 : 2.5). The crude product was purified by Shimadzu HPLC equipped with a Capcell pak ^{18}C column on a binary gradient system at a flow rate of 1.0 ml min^{-1} using an elution solvent of 0.1% trifluroacetic acid (TFA) in water (A) and 0.1% TFA in acetonitrile (B) with a gradient elution profile of (B) 0～10% in 2 min; 10～40% in 10 min; and 40～70% in 2 min. The molecular mass was analyzed on an LC-MS.

3 Radiochemistry of ^{177}Lu-DOTA-sBBNA

1) Preparation of ^{177}Lu-DOTA-sBBNA

Peptide was dissolved in a 50 mM sodium acetate buffer (pH=5.5) to give a concentration of 10^{-6} mole ml^{-1}. 37 MBq of a ^{177}Lu solution diluted in a 0.05 N HCl was injected into 1×10^{-8} mole of a peptide solution vial to give a final volume of 1 ml, and heated at 90˚C for 30 min. The radiolabeling yield and radiochemical purity/stability of the radiolabeled compound were analyzed through a Waters Chromatograph equipped with an X-Terra ^{18}C column. The column was eluted with a binary gradient system with a flow rate of 1.0 ml min^{-1} using an elution solvent of 0.1% TFA in 5% acetonitrile and 0.1% TFA in 95% acetonitrile. The gradient elution profile based on the solution of 0.1% TFA in 95% acetonitrile is as follows: 0%, 5 min; 0～100%, 9 min; 100%, 6 min; 100%, and 2 min with 100% of 0.1% TFA in 5%

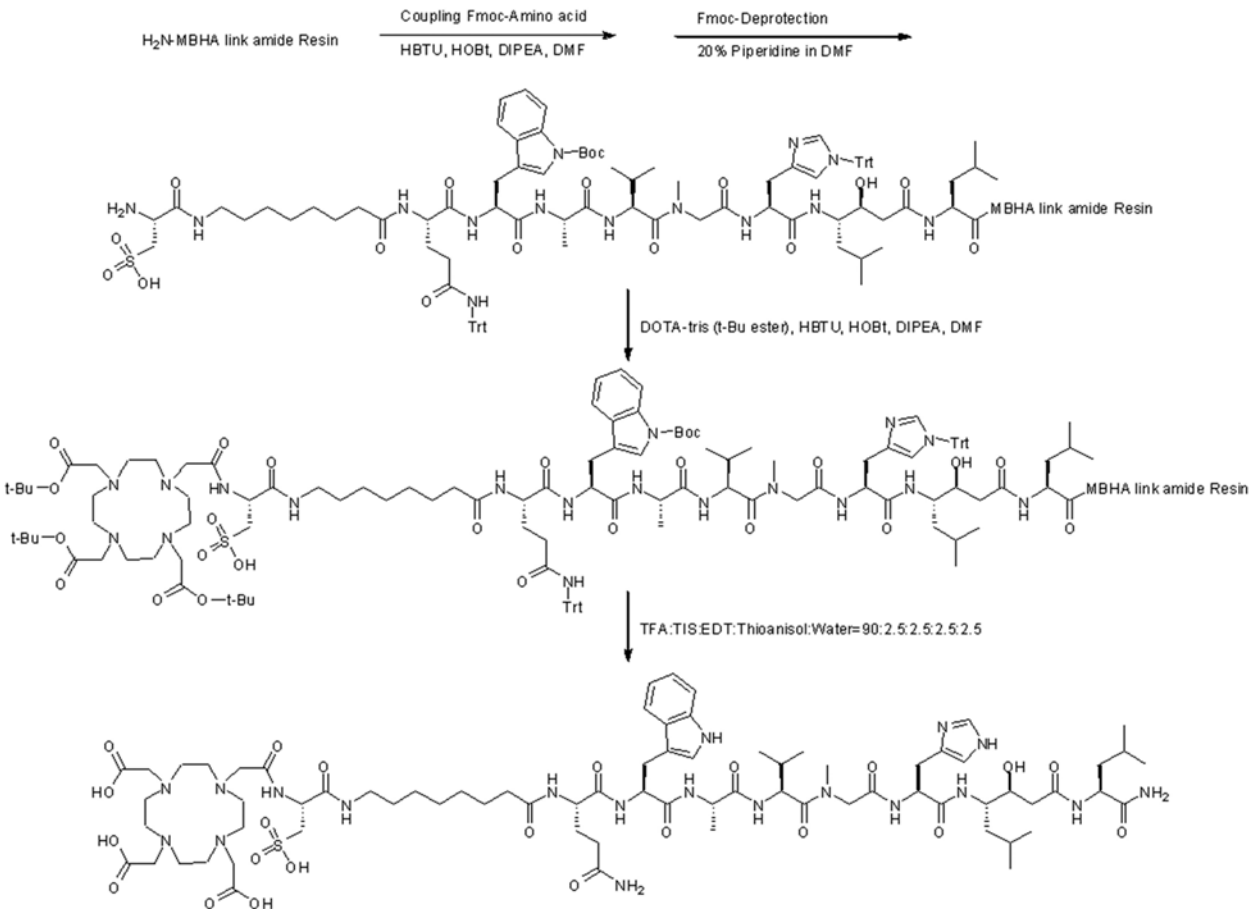

Fig. 1. Solid phase synthesis route and their formula of DOTA-sBBNA. A final sequence of DOTA-sBBNA was DOTA-Ala(SO_3H)-Aminooctanoyl-Gln-Trp-Ala-Val-N methyl Gly-His-Statine-Leu-Met-NH_2.

acetonitrile.

2) Determination of Log *P* value

37 KBq of ^{177}Lu-DOTA-sBBNA was dissolved in an equal volume mixture of 1-octanol and a PBS buffer (1 ml : 1 ml). After stirring vigorously for ~20 min, the mixture was centrifuged at a speed of 8,000 rpm for 5 min. 100 µl of samples from both 1-octanol and PBS layers were transferred and the radioactivity was measured using a Wallac 1470 Wizard automated gamma counter (PerkinElmer Life Science). Partition coefficients were measured three different times. The log *P* values were reported as the average of three independent measurements.

3) *In vitro* stability assay

The serum stability was evaluated as described by Nguyen *et al.* with some modification (Leonard et al. 2010). ^{177}Lu-DOTA-sBBNA was added to 200 µl of PBS and 25% human serum in PBS, and incubated at 37˚C for 2 days. 100 µl aliquots of the incubations were taken for the following time periods: 1 and 2 days. The aliquots were mixed with 40 µl of 15% trichloroacetic acid (TCA) and incubated at 4˚C for at least 15 min to precipitate the serum proteins. 5 µl of 1 M NaOH was supplemented to the TCA to prevent peptide precipitation. The supernatant was collected for each sample after centrifugation at 13,000 rpm for 10 min and analyzed by HPLC analysis, as described above.

4) Urinary metabolites of ^{177}Lu-DOTA-sBBNA

7.4 MBq of ^{177}Lu-DOTA-sBBNA was injected into Balb/c nude mice through the tail vein (n=4). After administration, the urinary samples were collected at 2 hr after injection using metabolic cages. 200 µl of radioactive metabolites in urine were mixed with 40 µl of 15% trichloroacetic acid (TCA) and incubated at 4˚C for 15 min to precipitate serum proteins. 5 µl of 1 M NaOH was supplemented to the TCA to

prevent peptide precipitation. The supernatant was collected for each sample after centrifugation at 13,000 rpm for 10 min and analyzed using HPLC analysis, as described above.

4. Biological evaluation of ^{177}Lu-DOTA-sBBNA

1) Cell culture

The GRPR-over-expressing PC-3 prostate carcinoma cells were obtained from the American Type Culture Collection (ATCC) and grown in 100 mm culture dishes (Corning, Corning, NY, USA). The cells were cultured in RPMI-1640 (LONZA, Walkersville, MD, USA), supplemented with 10% fetal bovine serum, 100 units ml^{-1} penicillin, and 100 g ml^{-1} streptomycin (Sigma, Milan, Italy) in an atmosphere of 5% CO_2 in air at 37°C for up to approximately a 90% confluence.

2) Competitive binding assay

The IC_{50} values of the peptide was determined using previously described methods with some modifications (Yubin Miao 2008). 1×10^5 PC-3 cells were placed in 12-well plates, and grown for 24 h at 37°C. After replacing the culture media with a FBS free-RPMI-1640, the cells were incubated at 37°C for 1 hr with 20,000 cpm of ^{125}I-[Tyr^4]-BBS (Perkin-Elmer, USA) in the presence of increasing concentrations of the peptide ($10^{-6} \sim 10^{-12}$ M) in a 1 ml binding buffer. The reaction media were collected. Cells were then washed twice with a cold PBS and solubilized with 1 N NaOH for 5 min. The activity was then determined in a gamma-counter. The IC_{50} value for the peptide was calculated through a non-linear regression analysis using the GraphPad Prism5 computer fitting program.

RESULTS AND DISCUSSION

The DOTA-sBBNA was synthesized through a solid phase peptide synthesis in accordance with the Fmoc strategy. Fig. 1 shows the scheme of the synthesis and the final structural formula.

The retention time of the analytical HPLC for DOTA-sBBNA was found to be 6.02 min, and the chemical purity was over 98% (Fig. 2A). Fig. 2B shows that the measured ion peak [M+1 (m/z)] was found at 1658, which is consistent with the proposed formula (Calculated=1657.40). The final peptide sequence of DOTA-sBBNA was DOTA-Ala(SO_3H)-Aminooctanoyl-Gln-Trp-Ala-Val-N methyl Gly-His-Statine-Leu-NH_2.

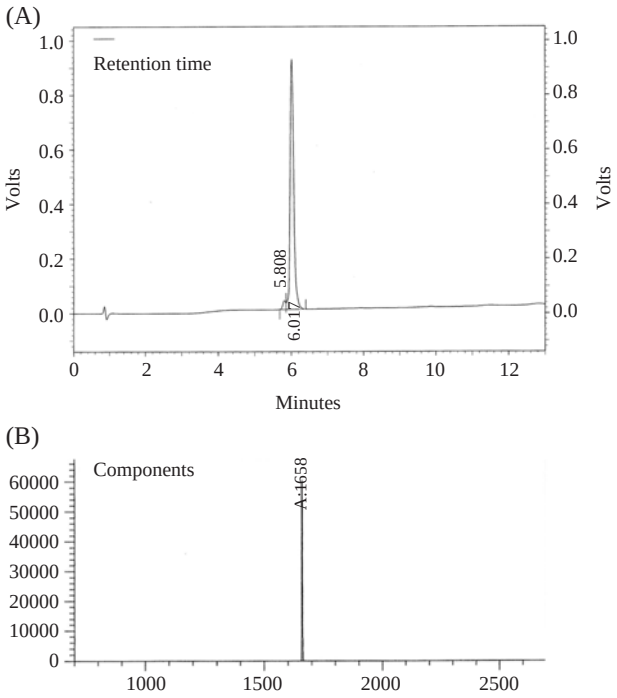

Fig. 2. HPLC analysis (A) and LC/MS profiles (B) of DOTA-sBBNA. The crude product was purified by Shimadzu HPLC equipped with a Capcell pak ^{18}C column and the molecular mass was analyzed on LC-MS. A purity of the peptide was over 98%, and a final MS data of the peptide was equal to the calculated value of the proposed formula.

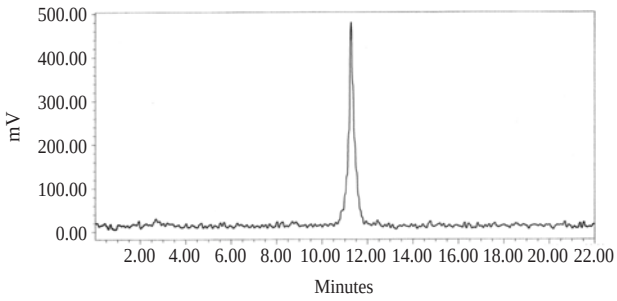

Fig. 3. Typical profiles of ^{177}Lu-DOTA-sBBNA determined by HPLC analysis using a ^{18}C column. Radiochemical purity of the peptides was over 98% and further purification was not needed. Retention time : 11.29 min.

For ^{177}Lu radiolabeling, the peptides were mixed with a ^{177}Lu solution and heated at 90°C for 30 min as described in the methods. A gamma detector-equipped HPLC analyzer was used to evaluate the labeling yield of the peptides, and the results are shown in Fig. 3. A high labeling yield (>98%) was achieved, and used directly without further purification.

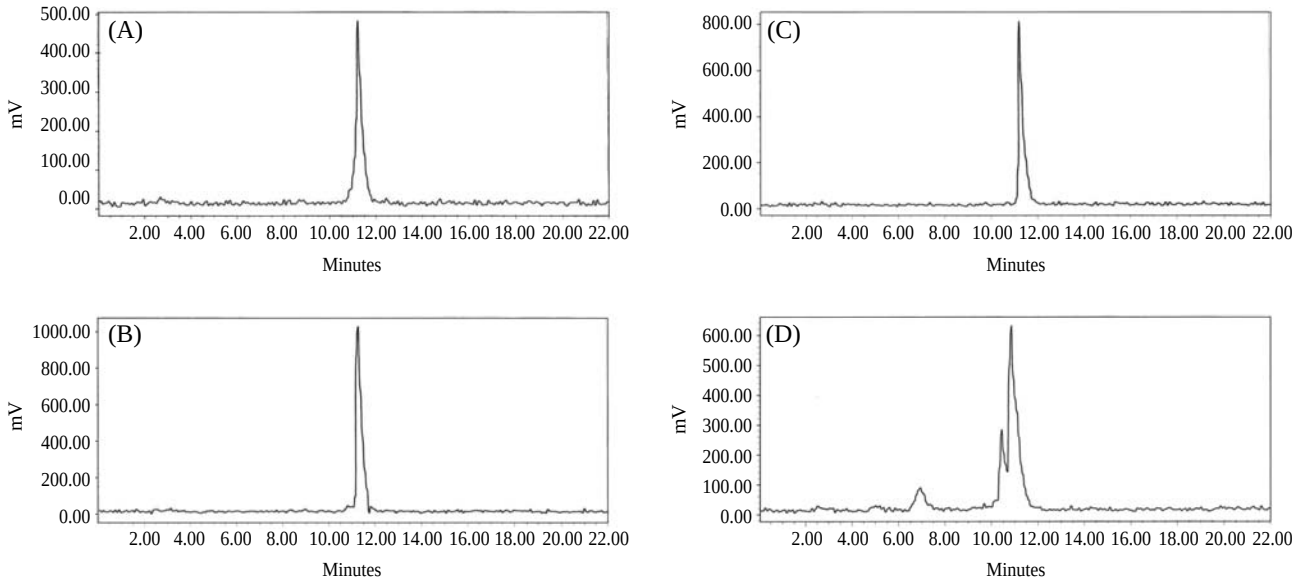

Fig. 4. *In vitro* stability of ^{177}Lu-DOTA-sBBNA in PBS (A) and human serum (B). Stock solution (C) and *in vivo* metabolism of radiolabeled peptide in murine urine (D). The radiolabeled peptide was stable both in PBS and human serum for 48 hr. It was unstable in murine *in vivo*, and 3 main metabolites were detected in murine urine at 15 minutes after an injection of the radiolabeled peptide.

Because a DOTA chelator can be radiolabeled with a lot of radionuclides such as ^{68}Ga, ^{90}Y, ^{177}Lu, and ^{111}In, it is encouraged to apply the peptide for the imaging and treatment with other radionuclides (Lim *et al.* 2012).

The Log *P* value of the ^{177}Lu-DOTA-sBBNA was determined through a shake flask method. This value was found to be −2.05. Increasing the hydrophilicity as well as the overall charge of the bombesin analog may lead to derivatives with improved sensitivity, specificity, and pharmacokinetics for the optimal targeting of GRPR-positive tumors. Log *P* value of ^{18}F-BAY 86-4367 was −3.9 indicating highly hydrophilic, and it showed more specific and effective GRPR-based targeting *in vivo*. In addition, rapid tumor targeting and fast renal excretion (~70%) and hepatobiliary excretion (~10%) were identified in PC-3 xenograft models (Honer *et al.* 2011). Christian *et al.* also demonstrated that an introduction of hydrophilic triazole coupled glucose into the ^{99m}Tc labeled BBS analogue resulted in a reduction of the abdominal accumulation and an improvement of the tumor-to-background ratios (Schweinsberg *et al.* 2008). The hydrophilicity of the peptide plays an important role in the targeting affinity *in vitro* as well as in the biodistribution *in vivo*. Although several reports suggest that higher hydrophilicity is advantageous for targeting GRPR, the injected radiolabeled peptide might excreted too rapid to be accumulated in the targeted organ. Additionally, an unwanted radiation dose might be delivered to the kidneys because hydrophilic compounds are likely to be retained there.

The ^{177}Lu-DOTA-sBBNA did not show any degradation in either PBS or human serum up to 48 hr (Fig. 4A, B). Michael *et al.* reported the stability of the bombesin antagonist-based peptide, ^{18}F-BAY 86-4367. The ^{18}F-labeled peptide was stable for 2 hours both in PBS and human plasma. It is encouraged to apply radionuclide therapy because the higher serum stability can make a higher accumulation of the radiolabeled peptide in the targeted organ and deliver more radiation dose to the organ.

^{18}F-BAY 86-4367 was unstable in murine plasma and 1 polar metabolite was detected in just 15 minutes after an injection of the radiolabeled peptide. Additionally, all of the parent compounds were degraded, and 3 main metabolites were detected in murine urine. Although it is a little unstable in mice *in vivo*, ^{18}F-BAY 86-4367 clearly visualized PC-3 xenografted prostate tumor. The *in vivo* stability of ^{177}Lu-DOTA-sBBNA was also analyzed in murine urine by radio-HPLC (Fig. 4D). At 15 minutes after an injection of the radiolabeled peptide into nude mice, urine samples were collected for HPLC analysis. Similar to the ^{18}F-BAY 86-4367, 3 radiometabolites could be detected in the urine (Fig. 4B). Because HPLC conditions for analysis of the samples

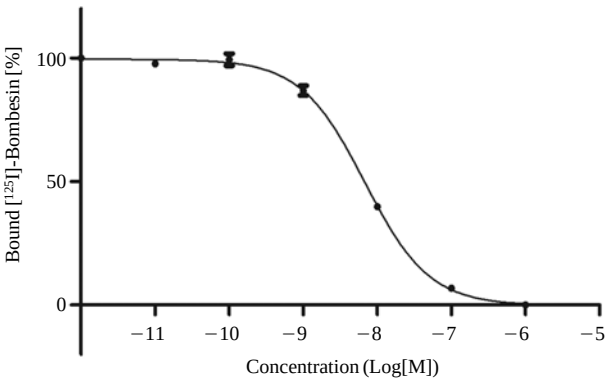

Fig. 5. Competitive binding of ^{125}I-[Tyr^4]-Bombesin on PC-3 cells by a treatment of DOTA-sBBNA. Results expressed as a percentage of binding are mean ± SD in triplicate. 1×10^5 PC-3 cells were incubated at 37°C for 1 hr with 20,000 cpm of ^{125}I-[Tyr^4]-Bombesin (Perkin-Elmer, USA) in the presence of increasing concentrations of the peptide ($10^{-6} \sim 10^{-12}$ M) in a 1 ml binding buffer. The IC_{50} of DOTA-sBBNA was 6.76 nM indicating higly nanomolar binding affinity for the GRPR.

were different, the peaks of the metabolites were detected at different retention times. However, all metabolites of ^{18}F-BAY 86-4367 and ^{177}Lu-DOTA-sBBNA were more hydrophilic than the parent radiolabeled peptide, and the number of main metabolites were 3, respectively (Honer *et al.* 2011). As 2 peptides shared the same targeting sequence and a peak of ^{177}Lu was not detected in urine, it seems that the targeting sequence of the antagonist can be degraded *in vivo* and more studies to identify its cleavage sites are needed to improve the *in vivo* stability.

Compared with the reference peptide ^{18}F-BAY 86-4367 (IC_{50}=0.94 nM), DOTA-sBBNA still retained reasonable affinity to the GRPR (IC_{50}=6.76 nM), as shown in Fig. 5. The main differences between two radiolabeled peptides are the chelator and linker moiety. The insertion of more hydrophilic moiety, Ala(SO_3H) into its linker made the binding affinity higher in the case of ^{18}F-BAY 86-4367 (Honer *et al.* 2011). Because the hydrophilicity of the ^{177}Lu-DOTA-sBBNA is still much lower than ^{18}F-BAY 86-4367, an insertion of more Ala(SO_3H) or hydrophilic carbohydrate into the linker might make its binding affinity higher.

Another BBS antagonist using a DOTA chelator is DOTA-AR, and its targeting sequence was D-Phe-Gln-Trp-Ala-Val-Gly-His-Sta-Leu-NH_2. Although IC_{50} of it was 18 nM, which was lower than DOTA-sBBNA, 10.56 ± 0.70%ID g^{-1} of the peptide was accumulated in PC-3 xenografted tumor at 1 hr p.i.. NODAGA-AR even showed a lower binding affinity (IC_{50}=25 nM), but it was labeled with ^{68}Ga and successfully detected a PC-3 tumor in PET/CT imaging (Abiraj *et al.* 2011). The same targeting sequence was conjugated with a DOTA via the cationic spacer 4-amino-1-carboxymethyl-piperidine, which was named RM2. The exchange of linker moiety resulted in an alteration of hydrophilicity and an increase of its IC_{50} (7.7 nM). Radiolabeled RM2 showed a high accumulation in PC-3 tumors, which was 15.2 ± 4.8 %ID g^{-1} at 1 h p.i. (Mansi *et al.* 2011). Therefore, the strategy to modify a linker moiety chemically might be effective, and the targeting affinity of DOTA-sBBNA can be improved by a change in its linker moiety. In addition, Renzo *et al.* reported that the Desmobesin showed the highest PC-3 tumor accumulation *in vivo*. BBS antagonist sequence, [D-Phe^6, Leu-NHEt^{13}, des-Met^{14}] bombesin$_{6-14}$ was used for targeting GRPR, and 24.61 ± 1.98%ID g^{-1} was accumulated in a PC-3 tumor at 1 hr p.i. (Cescato *et al.* 2008). Further studies to improve the binding affinity should consider the targeting moiety used in Desmobesin.

A series of results suggest that ^{177}Lu-DOTA-sBBNA has a promising characteristic as a modality to treat the GRPR-over-expressing tumors. ^{177}Lu-DOTA-sBBNA can deliver a sufficient radiation dose to the targeted organ owing to its high serum stability and binding affinity. Therefore, we plan to evaluate its pharmacokinetic characteristics and therapeutic efficacy in the next study.

CONCLUSION

In conclusion, a novel GRPR-binding peptide antagonist, ^{177}Lu-DOTA-Ala(SO_3H)-Aminooctanoyl-Gln-Trp-Ala-Val-N methyl Gly-His-Statine-Leu-NH_2, is a promising candidate for the targeting of GRPR-over-expressing tumors, and further investigations to evaluate its pharmacokinetic characteristics and therapeutic efficacy are needed.

ACKNOWLEDGEMENTS

This study was supported by the KAERI Major Project, Development of Radioisotope Production and Application Technology based on Research Reactor (525140-13).

REFERENCES

Abd-Elgaliel WR, Gallazzi F, Garrison JC, Rold TL, Sieckman

GL, Figueroa SD, Hoffman TJ and Lever SZ. 2008. Design, synthesis, and biological evaluation of an antagonist-bombesin analogue as targeting vector. *Bioconjug. Chem*. **19**(10): 2040-2048.

Abiraj K, Mansi R, Tamma ML, Fani M, Forrer F, Nicolas G, Cescato R, Reubi JC and Maecke HR. 2011. Bombesin antagonist-based radioligands for translational nuclear imaging of gastrin-releasing peptide receptor-positive tumors. *J. Nucl. Med*. **52**(12):1970-1978.

Bodei L and Nunn A. 2007. ^{177}Lu-AMBA bombesin analogue in hormone refractory prostate cancer patients patients : A phase i escalation study with single-cycle administrations [abstract]. *Eur. J. Nucl. Med. Mol. Imaging* **34**(suppl 2): S221.

Casanueva FF, Perez FR, Casabiell X, Camina JP, Cai RZ and Schally AV. 1996. Correlation between the effects of bombesin antagonists on cell proliferation and intracellular calcium concentration in swiss 3t3 and ht-29 cell lines. *Proc. Natl. Acad. Sci. U.S.A*. **93**(4):1406-1411.

Cescato R, Maina T, Nock B, Nikolopoulou A, Charalambidis D, Piccand V and Reubi JC. 2008. Bombesin receptor antagonists may be preferable to agonists for tumor targeting. *J. Nucl. Med*. **49**(2):318-326.

Depalatis FK, Cheng RC and Kotite NJ. 1995. Lysine reduces renal accumulation of radioactivity associated with injection of the [^{177}Lu]-[2-(4-aminophenyl)ethyl]-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid-cc49 Fab radioimmunoconjugate. *Cancer Res.* **55**:5288-5295.

Fleischmann A, Waser B and Reubi JC. 2007. Overexpression of gastrin-releasing peptide receptors in tumor-associated blood vessels of human ovarian neoplasms. *Cell Oncol*. **29**(5):421-433.

Gugger M and Reubi JC. 1999. Gastrin-releasing peptide receptors in non-neoplastic and neoplastic human breast. *Am. J. Pathol*. **155**(6):2067-2076.

Honer M, Mu L, Stellfeld T, Graham K, Martic M, Fischer CR, Lehmann L, Schubiger PA, Ametamey SM, Dinkelborg L, Srinivasan A and Borkowski S. 2011. ^{18}F-labeled bombesin analog for specific and effective targeting of prostate tumors expressing gastrin-releasing peptide receptors. *J. Nucl. Med.* **52**(2):270-278.

Kukis DS, Denardo GL, O'donnell RT and Meares CF. 1998. Optimized conditions for chelation of ^{90}Y-dota immunoconjugates. *J. Nucl. Med.* **39**:2105-2110.

Kwekkeboom KP, Bakker WH, Macke HR and Krenning EP. 1999. Comparison of ^{111}In-dota-tyr^{3}-octreotide and ^{111}In-DOTA-octreotide in the same patients: Biodistribution, kinetics, organ and tumor uptake. *J. Nucl. Med*. **40**:762-767.

Leonard N, Nicole Perry, Leonie DB, Sebastian AJ and Hans JV. 2010. Serum stabilities of short tryptophan- and arginine-rich antimicrobial peptide analogs. *Plus One* **5**(9):1-8.

Lim JC, Hong YD, Kim JJ, Choi SM, Baek HS and Choi SJ. 2012. Synthesis and biological evaluation of a novel ^{177}Lu-DOTA-[gly^{3}-cyclized (dap^{4}, d-phe^{7}, asp^{10})-arg^{11}]alpha-msh$_{3-13}$ analogue for melanocortin-1 receptor-positive tumor targeting. *Cancer Biother Radiopharm* **27**(8):464-472.

Mansi R, Wang X, Forrer F, Kneifel S, Tamma ML, Waser B, Cescato R, Reubi JC and Maecke HR. 2009. Evaluation of a 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid-conjugated bombesin-based radioantagonist for the labeling with single-photon emission computed tomography, positron emission tomography, and therapeutic radionuclides. *Clin. Cancer Res*. **15**(16):5240-5249.

Mansi R, Wang X, Forrer F, Waser B, Cescato R, Graham K, Borkowski S, Reubi JC and Maecke HR. 2011. Development of a potent dota-conjugated bombesin antagonist for targeting grpr-positive tumours. *Eur. J. Nucl. Med. Mol. Imaging* **38**(1):97-107.

Markwalder R and Reubi JC. 1999. Gastrin-releasing peptide receptors in the human prostate: Relation to neoplastic transformation. *Cancer Res*. **59**(5): 1152-1159.

Miao Y, Hoffman TJ and Quinn TP. 2005. Tumor-targeting properties of ^{90}Y and ^{177}Lu-labeled alpha-melanocyte stimulating hormone peptide analogues in a murine melanoma model. *Nucl. Med. Biol*. **32**(5):485-493.

Mu L, Honer M, Becaud J, Martic M, Schubiger PA, Ametamey SM, Stellfeld T, Graham K, Borkowski S, Lehmann L, Dinkelborg L and Srinivasan A. 2010. In vitro and in vivo characterization of novel ^{18}F- labeled bombesin analogues for targeting grpr-positive tumors. *Bioconjug. Chem*. **21**(10): 1864-1871.

Okarvi SM. 2004. Peptide-based radiopharmaceuticals: Future tools for diagnostic imaging of cancers and other diseases. *Med. Res. Rev*. **24**(3):357-397.

Pippin MT and Brechbiel MW. 1995. Lead (ii) complexes of 1,4,7,10-tetraazacyclododecane-n,n′,n″,n‴-tetraacetate: Solution chemistry and application to tumor localization with ^{203}pb labeled monoclonal antibodies. *Inorg. Chem. Acta.* **239**:43-51.

Reubi JC, Korner M, Waser B, Mazzucchelli L and Guillou L. 2004. High expression of peptide receptors as a novel target in gastrointestinal stromal tumours. *Eur. J. Nucl. Med. Mol. Imaging* **31**(6):803-810.

Ruegg CL, Brechbiel MW, Mirzadeh S, Gansow OA and Strand M. 1990. Improved in vivo stability and tumor targeting of bismuth-labeled antibody. *Cancer Res*. **50**:4221-4226.

Schweinsberg C, Maes V, Brans L, Blauenstein P, Tourwe DA, Schubiger PA, Schibli R and Garcia G. 2008. Novel glycated [^{99m}Tc(CO)^{3}]-labeled bombesin analogues for improved

targeting of gastrin-releasing peptide receptor-positive tumors. *Bioconjug. Chem*. **19**(12):2432-2439.

Smith CJ, Volkert WA and Hoffman TJ. 2003. Gastrin releasing peptide (grp) receptor targeted radiopharmaceuticals: A concise update. *Nucl. Med. Biol*. **30**(8):861-868.

Smith CJ, Volkert WA and Hoffman TJ. 2005. Radiolabeled peptide conjugates for targeting of the bombesin receptor superfamily subtypes. *Nucl. Med. Biol*. **32**(7):733-740.

Yubin M, Haixun G and Thomas PQ. 2008. ^{111}In-labeled lactam bridge-cyclized r-melanocyte stimulating hormone peptide analogues for melanoma imaging. *Bioconjugate Chem.* **19**:539-547.

Manuscript Received: November 6, 2013
Revised: November 18, 2013
Revision Accepted: November 20, 2013