

# Preparation and evaluation of $^{67}\text{Ga}$ -DOTA-Bombesin (7-14) as a tumor scintigraphic agent

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## ABSTRACT

**Introduction:** Bombesin is a 14-aminoacid peptide isolated from frog skin. The mammalian counterparts of the frog peptide are neuromedin B (NMB) and gastrin-releasing peptide (GRP). Bombesin (BBN) is a peptide showing high affinity for the gastrin releasing peptide receptor (GRPr). Prostate, small cell lung cancer, breast, gastric, and colon cancers are known to over express receptors to bombesin (BBN) and gastrin releasing peptide (GRP). In this study a new  $^{67}\text{Ga}$  radiolabeled BBN analogue evaluated based upon the bifunctional chelating ligand DOTA (1, 4, 7, 10-tetraazacyclododecane-1, 4, 7, 10-tetraacetic acid) that can be used as a tool for diagnosis of GRP receptor-positive tumors.

**Methods:** DOTA-BBN (7-14) NH<sub>2</sub> was synthesized using a standard Fmoc strategy. Labeling with  $^{67}\text{Ga}$  was performed at 95°C for 30 minutes in ammonium acetate buffer (pH = 4.8). Radiochemical analysis involved ITLC and HPLC methods. The stability of radiopeptide was examined in the presence of human serum at 37°C up to 24 hours. The receptor-bound internalization and externalization rates were studied in GRP receptor expressing PC-3 cells. Biodistribution of radiopeptide was studied in nude mice bearing PC-3 tumor.

**Results:** Labeling yield of >90% was obtained corresponding to a specific activity of  $\approx 2.48$  MBq/nmol. Peptide conjugate showed good stability in the presence of human serum. The radioligand showed a good and specific internalization into PC-3 cells (14.13 $\pm$ 0.61% at 4 h). In animal biodistribution studies, a receptor-specific uptake of radioactivity was observed in GRP-receptor-positive organs. After 4 h, uptake in mouse pancreas was 1.08  $\pm$  0.29% ID/g (percentage of injected dose per gram of tissue).

**Conclusion:** These data show that [ $^{67}\text{Ga}$ ]-DOTA-Bombesin (7-14) NH<sub>2</sub> is a specific radioligand for gastrin-releasing peptide receptor positive tumors

**Key words:** Bombesin,  $^{67}\text{Ga}$ , DOTA, Tumor, PC-3 cells

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## INTRODUCTION

Many malignant human cancers over express different peptide hormone receptors on their cell surface. These receptors have become important and useful as targets for molecular imaging and targeted therapy of tumors. Several peptide receptors such as somatostatin, neurotensin and bombesin receptors have attracted considerable interest in recent years. Bombesin is a 14-aminoacid peptide isolated from frog skin. The mammalian counterparts of the frog peptide are neuromedin B (NMB) and gastrin-releasing peptide (GRP). Over-expression of receptors for both NMB and GRP have been reported to be found on the cell surfaces of several malignant tissues, particularly in the cases of prostate and breast cancer (1-3).

In autoradiographic study, Reubi and Markwalder found the GRP receptor to be expressed in high density on invasive prostate carcinomas and proliferative intraepithelial prostate lesions, whereas normal prostate tissue were GRP receptor negative (4, 5). These findings suggest that the GRP receptor can be used as a molecular basis for diagnosing and treating prostate tumors.

Up to now, many types of radiolabeled BBN analogues have been designed to target GRP receptor expressing tumors (6-16). For example,  $^{99\text{m}}\text{Tc}$ ,  $^{111}\text{In}$  and  $^{67}\text{Ga}$  labeled BBN analogues have been developed for SPECT and  $^{64}\text{Cu}$  and  $^{68}\text{Ga}$  labeled analogues for positron emission tomography (PET) imaging (4,17-25)  $^{90}\text{Y}$  and  $^{177}\text{Lu}$  labeled analogues have been described as promising tools for targeted radiotherapy of these tumors (4, 5).

We have recently developed and evaluated the radiolabeled peptide [ $^{99\text{m}}\text{Tc}/\text{HYNIC}^0$ , D-Tyr<sup>6</sup>, D-Trp<sup>8</sup>] bombesin (6-14)  $\text{NH}_2$  which internalized rapidly into GRP receptor positive tumor cells (26, 27). To extend our previous study, we extended our work to synthesize a DOTA coupled BBN analogue with improved affinity to GRP receptors and

increased uptake in GRP receptor expressing tumors.

Here we present data on the synthesis and labeling of DOTA-Bombesin (7-14)  $\text{NH}_2$  with a gamma and auger electron emitter  $^{67}\text{Ga}$ . In addition we studied stability in human serum, receptor bound internalization, efflux in PC-3 cells and in vivo tumor uptake and tissue biodistribution of radiolabeled compound.

## METHODS

Rink amide MBHA (4-Methylbenzhydrylamine) resin and all of the Fmoc-protected amino acids were commercially available from NovaBiochem (Laufelfingen, Switzerland). The prochelator 1,4,7,10-tetraazacyclododecane-1,4,7-tris(acetic acid-t-butyl ester)-10-acetic acid [DOTA-tris(tBu ester)] was obtained from Macrocylics (USA). Other reagents were purchased from Fluka, and used without further purification.

The reactive side chains of the amino acids were masked with one of the following groups: Trp, t-butoxycarbonyl; His, trityl; Gln, trityl. The cell culture medium was Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), amino acids, vitamins and penicillin/streptomycin from Gibco. The production of  $^{67}\text{Ga}$  was performed at a 30 MeV cyclotron (Cyclone-30, IBA) from cyclotron division, (AEOI). Analytical reverse phase high performance liquid chromatography (RP-HPLC) was performed on a JASCO 880-PU intelligent pump HPLC system equipped with a multiwavelength detector and a flow-through Raytest-Gabi  $\gamma$ -detector. CC 250/4.6 Nucleosil 120-5 C18 column from Teknokroma was used for analytical HPLC, and a VP 250/10 Nucleosil 100-5 C18 column was used for semipreparative HPLC. The gradient systems consisted of 0.1% trifluoroacetic acid/water (Solvent A)

and acetonitrile (Solvent B). For analytical HPLC, Gradient I was used: 0 min 95% A (5% B), 5 min 95% A (5% B), 25 min 0% A (100% B), 27 min 0% A (100% B), 30 min 95% A (5% B), flow = 1 mL/min,  $\gamma = 280$  nm; for semipreparative HPLC Gradient II: 0 min 80% A (20% B), 2 min 80% A (20% B), 17 min 50% A (50% B), 19 min 0% A (100% B), 21 min 0% A (100%B), 25 min 80%A (20% B), flow = 2 mL/min,  $\gamma = 280$  nm. Quantitative gamma counting was performed on an ORTEC Model 4001 M  $\gamma$ -system well counter.

### Synthesis

The peptide was synthesized by standard Fmoc solid phase synthesis on Rink Amide MBHA resin with substitution, 0.69 mmol/g. Coupling of each amino acid was performed in the presence of 3 mol excess of Fmoc-amino acid, 3 mol excess of N-hydroxybenzotriazole (HOBt), 3 mol excess of Diisopropylcarbodiimide (DIC) and 5 mol excess of diisopropylamine (DIPEA) in Dimethylformamide (DMF). Completeness of coupling reactions was monitored by the Kaiser test and the Fmoc groups were removed by adding 20% piperidine in DMF. Coupling of DOTA to peptide was performed in the presence of 1.2 mol excess of DOTA-(tBu)<sub>3</sub> 2.5 mol excess of (2-(7-Aza-1H-benzotriazole-1-yl)-1, 1, 3, 3-tetramethyluronium hexafluorophosphate) (HATU), 5 mol excess of diisopropylethylamine (DIPEA) in Dimethylformamide (DMF). The peptide DOTA conjugate was removed from the resin and amino acid side chains were also deprotected by treatment with a cocktail of trifluoroacetic acid (TFA), triisopropylsilane and water (95:2.5:2.5). After removing the organic solvents in vacuum, the crude product was precipitated with cold Petroleum ether and Diisopropyl ether (50:50). The crude peptide DOTA conjugate was dissolved in water/methanol and purified by semi-preparative RP-HPLC;

then the purified product was characterized by analytical HPLC.

### Labeling of DOTA-Bombesin (7-14) NH<sub>2</sub> with $^{67}\text{Ga}$

A stock solution of DOTA-Bombesin (7-14) NH<sub>2</sub> (concentration 1 mmol/l) was prepared by dissolving the peptide in distilled water. 15  $\mu\text{l}$  of the stock solution (20  $\mu\text{g}$  of peptide) was added to an eppendorf tube containing 0.2 ml ammonium acetate buffer (pH 4.8, 0.5 mol/l). 37 MBq [ $^{67}\text{Ga}$ ] in 0.02 ml/0.1 mol/l HCl was added to a reaction solution and mixture was kept for 30 min at 95°C. After cooling down to room temperature the preparation was checked for bound and free  $^{67}\text{Ga}$ .

### Characterization of $^{67}\text{Ga}$ -labeled DOTA-Bombesin (7-14) NH<sub>2</sub>

$^{67}\text{Ga}$ -labeled DOTA-Bombesin (7-14) NH<sub>2</sub> was characterized by Paper chromatography and HPLC techniques. Paper chromatography was performed using Whatman No.1 and methanol/0.01 mol/l acetate buffer (pH 6.2) at a ratio of 55:45 as a mobile phase to check for bound and free  $^{67}\text{Ga}$ . HPLC was used to ensure that the labeled molecule was present as a single peak and to determine the complexation yield. For Analytical HPLC a C-18 reversed phase column with gradient system 1 was used with 0.1% trifluoroacetic acid/water (Solvent A) and acetonitrile (Solvent B) as the mobile phase.

### Serum stability

To 1 mL of freshly prepared human serum, we added 1.49 nmol [ $^{67}\text{Ga}$ ]-DOTA-Bombesin (7-14) NH<sub>2</sub> and mixture was incubated in 37°C environment. At different time points, 100  $\mu\text{l}$  aliquots was removed and treated with 100  $\mu\text{l}$  of alcohol. Sample was centrifuged for 15 min at 3000 rpm to precipitate serum proteins. Supernatant was removed and activity in the supernatant

compared with the activity in sediment to give the percentage of radiopeptide or radiometal bound or transferred to the serum proteins. Supernatant was analyzed with HPLC Gradient I to determine the stability of labeled compound.

### Cell culture

The PC-3 cells were cultured in DMEM supplemented with 10% FBS, 2 mM glutamine and penicillin-streptomycin. Cells were maintained in a humidified 5%  $\text{CO}_2$ /air atmosphere at  $37^\circ\text{C}$ . For all cell experiments, the cells were seeded at a density of 1 million cells per well in 6-well plates and incubated overnight with internalization medium (DMEM with 1% FBS).

### Internalization and nonspecific membrane binding

Medium was removed from the 6-well plates contain PC-3 cells with density of 1 million cells per well and cells were washed once with 2 ml of internalization medium (DMEM with 1% FBS). Furthermore, 1.5 ml internalization medium was added to each well, and the plates were incubated at  $37^\circ\text{C}$  for about 1 h. Afterwards, about 6.5 kBq (2.5 pmol total peptide mass per well) was added to the medium, and the cells were incubated at  $37^\circ\text{C}$  for various time periods. To determine nonspecific membrane binding and internalization, we incubated cells with the radioligand in the presence of  $150 \mu\text{M}$  bombesin.

The cellular uptake was stopped at appropriate time periods (30 min, 1, 2 and 4 h) by removing medium from the cells and washing twice with 1 mL of ice-cold phosphate-buffered saline (PBS). An acid wash for 10 min with a glycine buffer (pH=2.8) on ice was also performed twice. This step was to distinguish between membrane-bound (acid releasable) and internalized (acid resistant) radioligand. Finally, the cells were treated with 1 N

NaOH. The culture medium and the receptor-bound and internalized fractions for both with and without cold peptide were measured radiometrically in a gamma counter.

### Externalization

For externalization studies, the PC-3 cells ( $10^6$ /well) were incubated with radioligand. After 2 h internalization at  $37^\circ\text{C}$  and 5%  $\text{CO}_2$ , the medium was removed and the cells were washed twice with 1 ml ice cold PBS. Acid wash for a period of 5 min twice with a glycine buffer of pH 2.8 was done to remove the receptor bound ligand. Cells were then incubated again at  $37^\circ\text{C}$  with fresh internalization medium. After different time points (15 min, 30 min, 1 h, 2 h and 4 h), the external mediums were removed for quantification of radioactivity in a gamma counter.

The cells were solubilized in 1 N NaOH and removed, and the internalized radioactivity was quantified in a gamma counter. The externalized fraction was expressed as percentage of the total internalized amount per 1 million cells.

### Biodistribution

Animal experiments were performed in compliance with the regulations of our institution and with generally accepted guidelines governing such work. An activity of 3.7 MBq (1.49 nmol) of  $^{67}\text{Ga}$ -DOTA-Bombesin (7-14)  $\text{NH}_2$  was injected via the femoral vein. In order to determine the non-specific uptake of the radiopeptides, in receptor-positive organs, a group of three animals were injected with 100  $\mu\text{g}$  cold peptide in 50  $\mu\text{l}$  saline as a co-injection with the radiopeptides (blocked animals). After 1, 4 and 24 h, the mice in groups of three animals were killed, organs of interest were collected, weighed and radioactivity was measured in a gamma-counter. The percentage of the injected dose per gram (% ID/g) was calculated for each tissue.

## RESULTS

### Synthesis

DOTA-Bombesin (7-14)  $\text{NH}_2$  was synthesized by Fmoc strategy supplying an overall yield of 53% based on the removal of the first Fmoc group after cleavage, purification and lyophilization (Figure 1). The composition and structural identity of purified DOTA-peptide was verified by analytical HPLC. The purity was 96.1% as confirmed by HPLC method.

### Radiolabeling

Radiochemical purity of  $^{67}\text{Ga}$ -DOTA-Bombesin (7-14)  $\text{NH}_2$  conjugate was evaluated by RP-HPLC using the gradient systems consisted of 0.1% trifluoroacetic acid/water (Solvent A) and acetonitrile (Solvent B). The labeling yield of  $^{67}\text{Ga}$ -DOTA-Bombesin (7-14)  $\text{NH}_2$  was  $95 \pm 1.25\%$  ( $n=3$ ), acquired via HPLC at a specific activity of 2.48 MBq/nmol. The HPLC elution times (Gradient I) were 4.27 min for  $^{67}\text{GaCl}_3$  and 15.05 min for  $^{67}\text{Ga}$ -DOTA-Bombesin (7-14)  $\text{NH}_2$  (Figure 2).

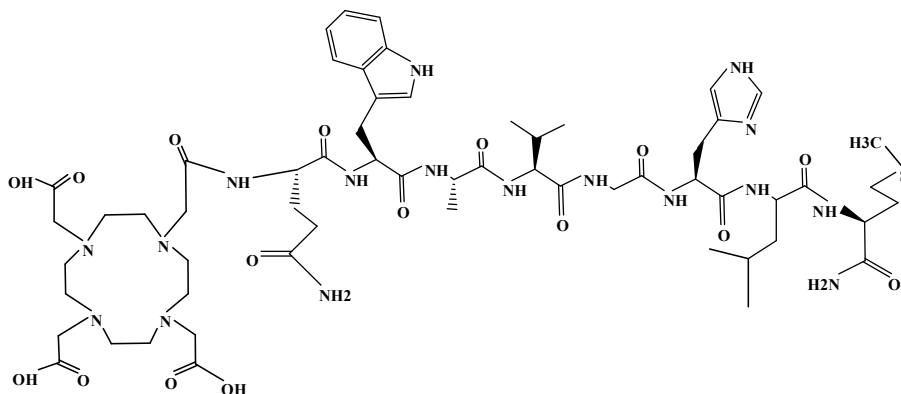


Fig 1. Structure of DOTA- Bombesin (7-14)  $\text{NH}_2$ , which could be labeled with  $^{67}\text{Ga}$ .

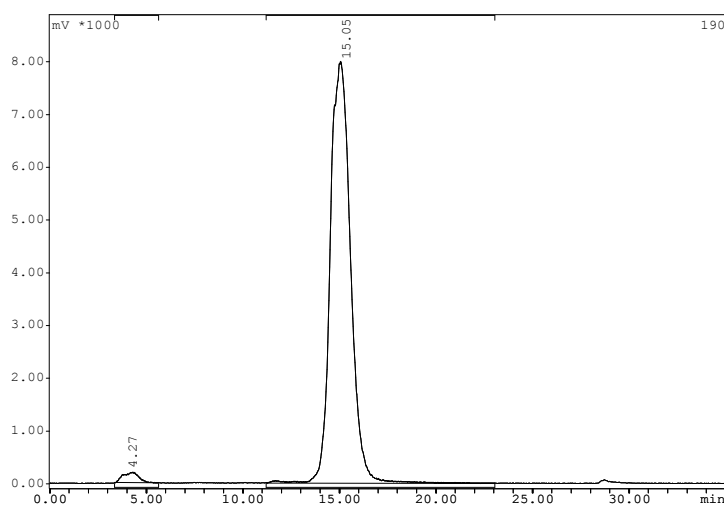
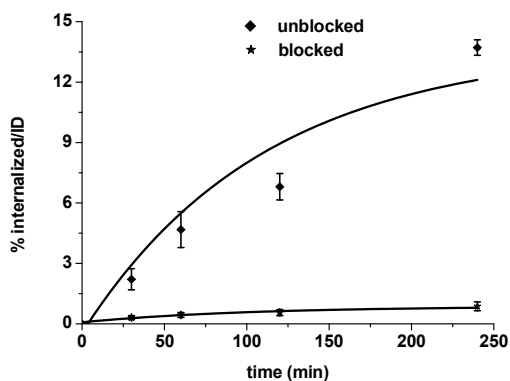


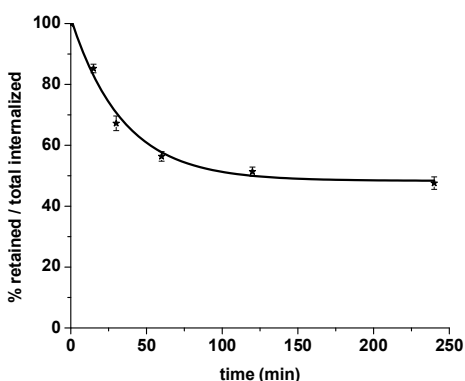
Fig 2. RP-HPLC profile of the  $^{67}\text{Ga}$ -DOTA-Bombesin (7-14)  $\text{NH}_2$

### Cell studies and stability

During 60 min, the radioligand showed  $4.98 \pm 0.85\%$  specific cell uptake, which increased to  $14.13 \pm 0.61\%$  up to 4 h (Figure 3). In all experiments, the internalization was strongly reduced in the presence of excess cold peptide. In fact, nonspecific internalization was  $0.78 \pm 0.31\%$  after 4 h, and the surface-bound peptide (acid removable) was  $0.97 \pm 0.23\%$  of the added activity after 4 h.



**Fig 3.** Internalization rate of  $^{67}\text{Ga}$ -DOTA-Bombesin (7-14)  $\text{NH}_2$  into PC-3 cells. Data are from three independent experiments with triplicates in each experiment and are expressed as specific internalization.



**Fig. 4.** Externalization over time for  $^{67}\text{Ga}$ -DOTA-Bombesin (7-14)  $\text{NH}_2$  in PC-3 cells.

After 15 min, for a 2-hour internalized radioligand,  $13.85 \pm 2.14\%$  of activity was externalized ( $86.15 \pm 2.41\%$  remained), which increased to  $51.42 \pm 2.58\%$  at 4 h ( $P < 0.05$ ). With more time, the percentage of externalization reached a plateau (Figure 4). Up to 24 h incubation in human serum the radiochemical purity was about 75% and there was not detected any major metabolite.

### Animal biodistribution

$^{67}\text{Ga}$ -DOTA-Bombesin (7-14)  $\text{NH}_2$  displayed rapid blood clearance with  $0.16 \pm 0.04$  ID/g at 4 hour (Table 1). Fast clearance from the gastrin-releasing peptide receptor-negative tissues except the kidneys was found as well. Labeled peptide show high uptake values in the gastrin-releasing peptide receptor-positive organs. By blocking the receptor through prior injection of cold peptide, the uptake in pancreas is diminished and this confirms the specificity of radioconjugate (Figure 5).

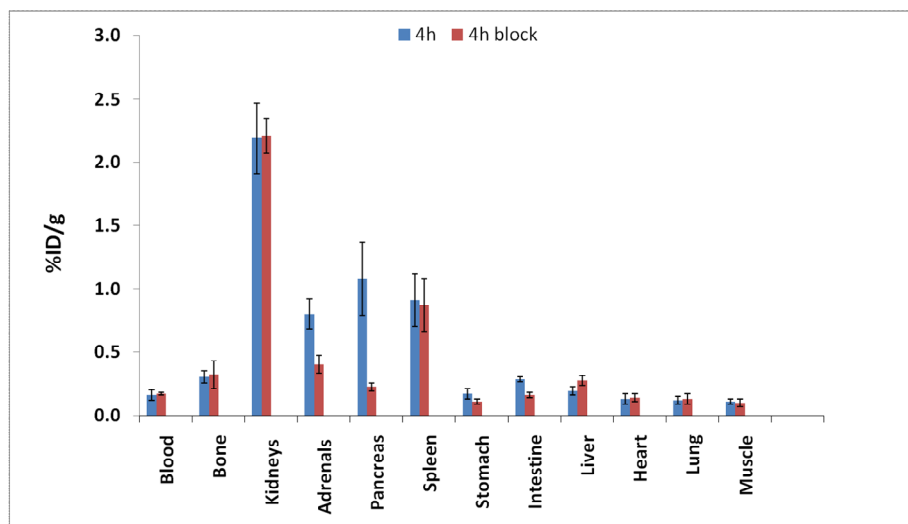
The percentages of reduction uptake was 79% ( $1.08\%$  ID/g vs.  $0.22\%$  ID/g at 4 h) respectively. On the other hand, the uptake reduction in non targeted tissues due to blocking dose was not significant.

## DISCUSSION

The successful application of radiolabeled somatostatin analogs in nuclear medicine for diagnostics and therapy of neuroendocrine tumors has stimulated the research in receptor targeting of additional tumor types (20). GRP receptors were shown to be over expressed on a variety of human tumors like breast and prostate cancer. Based on this fact and the experience with other peptides like somatostatin and ubiquicidin (28, 29) we concluded that targeting the GRP receptor with optimized analogue of bombesin is very important for scintigraphy of prostate and breast tumors.

**Table 1.** Biodistribution in mice. Data are presented as % injected dose per gram organ  $\pm$  SD,  $n = 3$ .

Organ	1 h	4 h	4 h block	24 h
Blood	0.27 $\pm$ 0.06	0.16 $\pm$ 0.04	0.17 $\pm$ 0.01	0.04 $\pm$ 0.01
Bone	0.36 $\pm$ 0.07	0.30 $\pm$ 0.05	0.32 $\pm$ 0.11	0.07 $\pm$ 0.01
Kidney	3.01 $\pm$ 0.26	2.19 $\pm$ 0.28	2.21 $\pm$ 0.14	0.53 $\pm$ 0.06
Adrenal	0.51 $\pm$ 0.09	0.8 $\pm$ 0.12	0.4 $\pm$ 0.07	0.52 $\pm$ 0.09
Pancreas	1.25 $\pm$ 0.12	1.08 $\pm$ 0.29	0.22 $\pm$ 0.03	0.57 $\pm$ 0.04
Spleen	0.72 $\pm$ 0.16	0.91 $\pm$ 0.21	0.87 $\pm$ 0.21	0.08 $\pm$ 0.02
Stomach	0.23 $\pm$ 0.05	0.17 $\pm$ 0.04	0.11 $\pm$ 0.02	0.09 $\pm$ 0.03
Intestine	0.45 $\pm$ 0.11	0.28 $\pm$ 0.02	0.16 $\pm$ 0.02	0.09 $\pm$ 0.03
Liver	0.29 $\pm$ 0.06	0.19 $\pm$ 0.03	0.27 $\pm$ 0.04	0.12 $\pm$ 0.04
Heart	0.18 $\pm$ 0.06	0.13 $\pm$ 0.04	0.14 $\pm$ 0.03	0.04 $\pm$ 0.01
Lung	0.25 $\pm$ 0.05	0.12 $\pm$ 0.03	0.13 $\pm$ 0.04	0.07 $\pm$ 0.02
Muscle	0.25 $\pm$ 0.04	0.11 $\pm$ 0.02	0.10 $\pm$ 0.03	0.04 $\pm$ 0.01

**Fig 5.** Biodistribution of  $^{67}\text{Ga}$ -DOTA-Bombesin (7-14)  $\text{NH}_2$  in mice 4 h after injection.

As we previously reported Bombesin analog [ $^{99\text{m}}\text{Tc}$ -HYNIC<sup>0</sup>, D-Tyr<sup>6</sup>, D-Trp<sup>8</sup>] bombesin (6-14) NH<sub>2</sub> is capable of visualizing GRP receptor positive tumors in vivo (26, 27). In the present study, we investigated a new DOTA coupled bombesin analogs with sequences bombesin (7-14) without any replacement. DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) was chosen as chelator since it chelates a large number of radiometals with high invitro and in vivo stability (30). DOTA chelator enables labeling with  $^{111}\text{In}$  and  $^{67}\text{Ga}$  for SPECT,  $^{68}\text{Ga}$  for PET imaging and  $^{177}\text{Lu}$  and  $^{90}\text{Y}$  as a beta emitter for radiotherapy of GRPr positive tumors.

The biological activity of [ $^{67}\text{Ga}$ ]-DOTA-Bombesin (7-14) NH<sub>2</sub> was determined through internalization and efflux studies in PC-3 cells. GRP receptors are belonging to G-protein-coupled receptors groups which after agonistic binding, go through endocytosis and internalization of the complex (9, 19, 20). High rate of internalization was observed for our compound (14.13±0.61% up to 4 h) which was not unexpected since Bombesin (7-14) NH<sub>2</sub> sequence offer agonistic property to compound. Beside efflux curve of [ $^{67}\text{Ga}$ ]-DOTA-Bombesin (7-14) NH<sub>2</sub> in PC-3 cells after 2 h of internalization showed an acceptable intercellular trapping. In contrast to our pervious works this new analog showed higher rate of internalization in compare with [ $^{99\text{m}}\text{Tc}$ /tricine/HYNIC<sup>0</sup>, D-Tyr<sup>6</sup>, D-Trp<sup>8</sup>] BN (6-14) NH<sub>2</sub> after 4 h in PC-3 cells (14.13±0.61% versus 10.7 ± 1.2%) (26, 27). An increased GRP receptor affinity and tumor uptake with replacement of DOTA instead of DTPA as a chelator in [pro1, tyr4] Bombesin analogue has also been reported (31). The choice of radionuclide also has an important role while the change in the M<sup>3+</sup> radiomethal used for DOTA labeling could alter the biodistribution of a DOTA conjugated peptide (32). Jochen Schuhmacher and coworker, found about 20% reduced uptakes

and retention of  $^{177}\text{Lu}$ -BZH3 in the tumor in comparison with [ $^{67}\text{Ga}$ ]-BZH3 (33). It has been shown that positive charge in sequence of peptide tends to interact faster and in a strong way with proteins, besides that is not targeted to a specific receptor (34). The cell internalization was receptor specific as was demonstrated with uptake results in GRPr blocked cell experiments which indicate the balance of charge for the complex.

In suitability of a radiopeptide for diagnostic or specially targeted radiotherapy an important aspect is stability in human serum which allows high concentration of intact radiopeptide for binding with receptors. Our bombesin analogue showed metabolic stability in human serum up to 24h after labeling and incubation. Results from Zhang et al. (4) show relatively low metabolic stability for [ $^{111}\text{In}$ ]-BZH1 and [ $^{111}\text{In}$ ]-BZH2. They found two degradation sites in their peptides sequences, one between  $\beta$ -Ala<sup>11</sup> and His<sup>12</sup> and another between Gln<sup>7</sup> and Trp<sup>8</sup>. Also in study by M. de Visser et al. (31) has been observed that changes in the bombesin amino acid sequence can have a marked effect on the peptides stability. They found that although substitution of native amino acids in bombesin sequences can enhance receptor affinity but not the serum stability.

Accumulation of radiopeptide in bombesin receptor positive tissues like the pancreas, the stomach, the intestines and adrenal was observed. The uptake in pancreas, intestine and adrenal was specific and receptor mediated, as shown by the co-injection of cold peptide, indicating that these organs are also GRP receptor positive.

The pancreas accumulation of this radioconjugate and good pharmacokinetic of radioligand like low tendency to accumulate in liver and intestine and high kidney excretion due to moderate lipophilicity are the major advantage of our compound. The ability for labeling of DOTA-Bombesin (7-14) NH<sub>2</sub> with  $\beta$  emitter radionuclides like  $^{90}\text{Y}$  and  $^{177}\text{Lu}$  is another advantage and is



important to formulate a useful therapeutic GRP receptor targeting radiopharmaceutical.

### CONCLUSION

In this study, we have shown synthesis and radio labeling of DOTA-Bombesin (7-14)  $\text{NH}_2$ .  $^{67}\text{Ga}$  labeled peptide was prepared with high yield at an acceptable specific activity of 2.48 MBq/ nmol which can be used as SPECT imaging agent. The radiolabeled conjugate was able to internalize in GRP receptor positive cancer cells. The prepared conjugate showed high accumulation in pancreas as a positive GRP receptors targeted tissues followed by excretion via the kidney. These promising characteristics make our new designed labeled peptide conjugate as a very suitable candidate for diagnosis or therapy of GRP receptor positive tumors in nuclear medicine.

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