

A lyophilized peptide radiopharmaceutical kit to target neurotensin receptor for tumor imaging

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ABSTRACT

Introduction: Neurotensin (NT) is a tridecapeptide that binds specifically to neurotensin receptors. Several forms of cancer, including small cell lung cancer, colon, pancreatic and prostate carcinomas especially exocrine pancreatic carcinomas express receptors for neurotensin peptide. Radiolabeled neurotensin derivatives with a high affinity for these receptors might be used for scintigraphy. The aim of this work was to prepare a freeze-dried kit formulation for routine preparation of ^{99m}Tc labeled neurotensin in nuclear medicine center.

Methods: A freeze dried kit containing reducing agent, coligands and HYNIC-Neurotensin derivative for labeling with ^{99m}Tc was prepared. Labeling was performed at 95°C for 15 min and radiochemical analysis involved ITLC and HPLC methods. The stability of radiopeptide was checked in the presence of human serum at 37°C up to 24 h. The receptor bound internalization rate was studied in neurotensin receptor expressing HT-29 cells. Biodistribution of radiopeptide was studied in mice.

Results: Labeling yield of >95% was obtained corresponding to a specific activity of 80 MBq/nmol. Prepared radioconjugate was stable in human serum and more than 12% of activity was specifically internalized into HT-29 cells up to 4 h. Biodistribution study showed a rapid blood clearance, with renal excretion and specific binding towards NT receptor-positive tissues such as intestines (0.99 ± 0.16% ID/g at 1 h).

Conclusion: The favorable characteristics of our new designed labeled peptide formulation make it as a promising candidate for diagnosis of malignant tumors.

Key words: HYNIC-neurotensin; ^{99m}Tc; Lyophilized kit; Tumor

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INTRODUCTION

Small receptors binding peptides are currently the agents of choice for receptor imaging and tumor targeting [1]. Several receptors such as somatostatin, bombesin and neurotensin receptors have attracted considerable interest in recent years [2]. Neurotensin (NT) is a tridecapeptide localized in central nervous system and in peripheral tissues. Three different receptor subtypes for neurotensin (NTR1, NTR2 and NTR3) have been described [3, 4]. Neurotensin receptors, in particular the first one with high affinity binding for NT, are expressed in several human tumors such as small cell lung cancer and colon, pancreatic and prostate carcinomas [5]. Due to the high incidence and density of neurotensin receptors in exocrine pancreatic carcinomas, neurotensin is considered the best possible candidate for peptide based diagnosis or therapy of these tumors [6]. Over 75% of all ductal pancreatic carcinomas were reported to overexpress neurotensin receptors, whereas normal pancreas tissue, pancreatitis and endocrine pancreas devoid of NT receptors [7]. Since pancreas carcinoma is among the most frequent causes of cancer deaths, the need to use new radiopharmaceuticals which are able to diagnose cancer in the early steps is evident.

Since ^{99m}Tc is still most ideal for diagnosis, based on its favorable nuclear properties, ^{99m}Tc based conjugates of neurotensin and other peptide derivatives continue to be attractive. Recently, several new NT conjugates radiolabeled with ^{99m}Tc have been reported [8]. Radiolabeling has been performed indirectly via a ligand including N_3S [9], N_4 [10], Hydrazino nicotinic acid (HYNIC) [9] and carbonyl [11]. Some of the above labeling procedures meet with such limitations as low radiochemical yield, extended reaction time, high temperature, high pH values, and high lipophilicity of the radiometal chelate. For example in ^{99m}Tc -carbonyl approach, although ^{99m}Tc -carbonyl commercial kits currently are readily available, consideration of the two steps preparation and the further purification of the final product seem to be unsuitable for its routine uses in the nuclear medicine centers. The use of the ^{99m}Tc -HYNIC core was first reported for the labeling of IgG and since then, has been conjugated to various biomolecules including antibodies and peptides [12]. Usually a co-ligand, such as tricine or ethylenediamine diacetic acid (EDDA), is included in the ^{99m}Tc labeling of HYNIC conjugates. In labeling process coligands tricine and EDDA can be used separately or together via exchange labeling method.

Teodoro et al [9] recently evaluated the radiochemical and biological behavior of NT (8-13) analogue radiolabeled with ^{99m}Tc , using HYNIC and NHS-S-acetyl-MAG₃ as chelator agents. They demonstrated that HYNIC seems to be a good choice

since a very high stability as well as a predominantly renal excretion was observed. We also recently reported the evaluation of a new somatostatin and bombesin analogues labeled via bifunctional chelating agents HYNIC and EDDA/tricine as coligands [13]. In continuation to our efforts to create a new ^{99m}Tc -labelled peptide for tumor targeting, we chose a NT (8-13) and a Gly⁶ as a spacer between HYNIC and N-terminus of the peptide. We also modified Lys⁹ and D-Tyr¹¹ instead of Arg⁹ and Tyr¹¹ respectively to decrease enzymatic metabolism.

Here we present data on the preparation of a freeze-dried kit formulation for routine preparation of ^{99m}Tc labeled neurotensin analogue in nuclear medicine centers. Optimum conditions for radiolabeling of conjugate with ^{99m}Tc , stability in human serum, receptor bound internalization in HT-29 cells and tissue biodistribution and scintigraphy for this freeze dried formulation were studied.

METHODS

2-Chlorotriyl chloride resin and all of the Fmoc-protected amino acids were commercially available from NovaBiochem (Laufelfingen, Switzerland). The prochelator HYNIC-Boc was synthesized according to Abrams et al. [12]. 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: Lys, tert-butoxycarbonyl (Boc); Tyr, tert-butyl (tBu); Arg, 2,2,4,6,7-pentamethyl-dihydrobenzofurane-5-sulfonyl (pbf). The cell culture medium was Roswell Park Memorial Institute (RPMI-1640) supplemented with 10% fetal bovine serum (FBS), amino acids, vitamins and penicillin/streptomycin from Gibco. Sodium pertechnetate ($\text{Na}^{99m}\text{TcO}_4$) obtained from commercial $^{99}\text{Mo}/^{99m}\text{Tc}$ generator (Radioisotope Division, Atomic Energy Organization of Iran). 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 γ -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, λ = 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,

$\lambda = 280$ nm. Mass spectrum was recorded on an Agilent 1100/ Bruker Daltonic (Ion trap) VL instrument (LC/MS). Quantitative gamma counting was performed on an EG&G / ORTEC (Ametek; Advanced Measurement Technology Division) Model 4001M Mini Bin & Power Supply counter.

Synthesis

The peptide was synthesized by standard Fmoc solid phase synthesis on 2-chlorotriyl chloride resin with substitution, 1.4 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). Completion of coupling reactions was monitored by the Kaiser test and the Fmoc groups were removed by adding 20% piperidine in DMF. Finally, coupling of Boc-HYNIC to peptide was performed in the presence of 1.2 mol excess of Boc-HYNIC, 2.5 mol excess of 2-(7-aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU), and 5 mol excess of DIPEA in DMF. The cleavage step from the resin and the final deprotection of all remained protecting groups was done in a standard cocktail containing trifluoroacetic acid (TFA), triisopropylsilane (TIPS), thioanisole, and water (92.5: 2.5: 2.5: 2.5). The crude peptide was precipitated into cold petroleum ether/diisopropyl ether (50:50). Then, the precipitate was dissolved in water/methanol (50:50) and purified by semi-preparative RP-HPLC (method II). The purified product was lyophilized in vacuum and characterized by analytical HPLC and LC-MS.

Kit formulation and Labeling with ^{99m}Tc

A stock solution of HYNIC-peptide (concentration 1 mg/mL) was prepared by dissolving the peptide in distilled water. 20 μL of this solution, 15 mg of tricine and 5 mg of EDDA in 0.5 mL of water were combined in a vial. To this solution was added 20 μL of 2 mg/mL $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ in nitrogen-purged 0.1 M HCl and then it was freeze-dried. For radiolabeling, 370-1,110 MBq of $^{99m}\text{TcO}_4^-$ in 0.5 mL saline was added to the lyophilized vial and incubated for 10 min at 95°C. After cooling down to room temperature the preparation was checked for bound and free ^{99m}Tc .

Radiochemical analysis

^{99m}Tc -labeled HYNIC-peptide was characterized by analytical RP-HPLC (gradient I) and ITLC on silica gel 60 (Merck) using different mobile phases: 2-butanone for free $^{99m}\text{TcO}_4^-$ ($R_f = 1$), 0.1 M sodium citrate (pH = 5) to determine the non-peptide bound

^{99m}Tc coligand and $^{99m}\text{TcO}_4^-$ ($R_f = 1$) and methanol/1 M ammonium acetate 1/1 for ^{99m}Tc colloid ($R_f = 0$). The radioactivity was quantified by cutting the strip ($1.5 \times 10 \text{ cm}^2$) into 1 cm pieces and counting in a well type gamma counter.

In vitro stability

Eighty six MBq of ^{99m}Tc -HYNIC-peptide was added to 1 ml of freshly prepared human serum, and the mixture was incubated in a 37°C environment. At different time points, 100 μL aliquots was removed and treated with 100 μL of alcohol. Sample was centrifuged for 5 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 binding and Internalization

The HT-29 cells were cultured in RPMI-1640 supplemented with 10% FBS, 2 mM glutamine and penicillin-streptomycin. Cells were maintained in a humidified 5% CO_2 /air atmosphere at 37 °C. To a 6-well plates contain HT-29 cells with density of 1 million cells per well, about 150 kBq of radioligand per well was added and the cells were incubated at 37 °C for various time periods. To determine nonspecific membrane binding and internalization, we incubated cells with the radioligand in the presence of 150 μL , 1 $\mu\text{mol/L}$ neurotensin. 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. 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.

Biodistribution

Animal experiments were performed in compliance with the regulations of our institution and with generally accepted guidelines governing such work. Healthy female Swiss mice (20-30 g) received 20 MBq of high specific activity radiotracer in 0.1 ml of saline via a tail 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 μg neurotensin in 50 μL 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. At 4 h after injection, accumulation of the tracer was also assessed by planar scintigraphy under ether anesthesia.

RESULTS

Synthesis

[HYNIC⁰, Gly⁷, Lys⁹, D-Tyr¹¹]-Neurotensin (7-13) was synthesized by Fmoc strategy supplying an overall yield of 40% based on the removal of the first Fmoc group after cleavage, purification and lyophilization (Figure 1). The composition and structural identity of purified HYNIC-peptide was verified by analytical HPLC and LC-MS. The purity was >98% as confirmed by HPLC method.

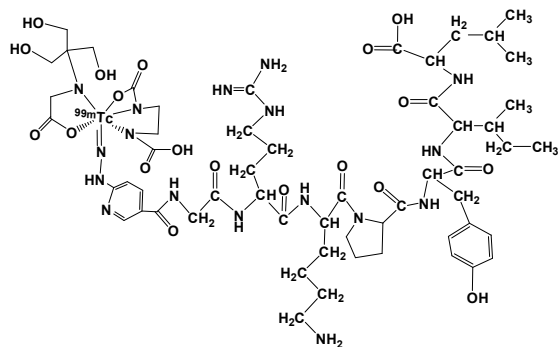


Fig 1. Proposed structure of ^{99m}Tc -tricine-EDDA-HYNIC-peptide.

Radiolabeling

Radiochemical purity of radiopharmaceutical kit 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 was >95%, acquired via HPLC and also ITLC at a specific activity of 81 MBq/nmol. The HPLC elution times (Gradient I) were 1.91 min for free pertechnetate and 16.85 min for labeled radiopharmaceutical kit (Figure 2).

Stability

The labeled radiopharmaceutical formulation was found to be stable in aqueous solution even after a 24 hrs period. In serum stability studies, $22.2 \pm 1.56\%$ of the activity was associated with the precipitate obtained after ethanol addition, indicating the low binding of complex with serum proteins. The ethanol fraction was characterized by HPLC where a single peak was observed at the same time (> 95%) as that of the complex, indicating no decomposition of the complex.

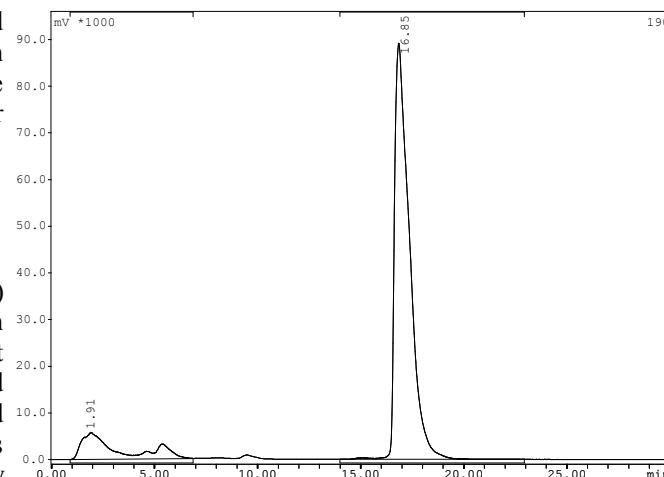


Fig 2. RP-HPLC profile of the radiopharmaceutical kit after labeling with ^{99m}Tc . The elution time for $^{99m}\text{TcO}_4^-$ is 1.91 min and for labeled radiopharmaceutical kit is 16.85 min (labeling yield 97.1%).

In vitro internalization

Figure 3 shows the result in respect of the time-dependent and specific internalization of the radiopharmaceutical into HT-29 cells. During 60 min, the radioligand showed $3.15 \pm 0.52\%$ specific cell uptake, which increased to $12.13 \pm 0.35\%$ up to 4 h. In all experiments, the internalization was strongly reduced in the presence of excess cold. In fact, nonspecific internalization was $0.69 \pm 0.21\%$ of the added activity after 4 h.

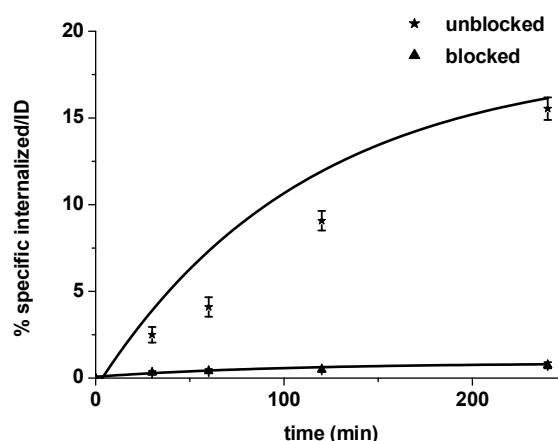


Fig 3. Internalization rate of radiolabeled kit into HT-29 cells. Data are from three independent experiments with triplicates in each experiment and are expressed as specific internalization.

Table 1. Biodistribution in mice (% injected dose per gram organ \pm SD, n = 3).

Organ	1 h	1 h block	2 h	4 h
Blood	0.40 \pm 0.11	0.43 \pm 0.13	0.18 \pm 0.03	0.12 \pm 0.02
Bone	0.26 \pm 0.07	0.24 \pm 0.05	0.05 \pm 0.06	0.07 \pm 0.02
Kidneys	8.17 \pm 0.75	7.39 \pm 0.58	3.65 \pm 0.22	2.41 \pm 0.19
Thyroid	0.41 \pm 0.10	0.42 \pm 0.21	0.33 \pm 0.11	0.26 \pm 0.05
Spleen	0.53 \pm 0.13	0.45 \pm 0.12	0.11 \pm 0.03	0.13 \pm 0.03
Stomach	0.53 \pm 0.12	0.42 \pm 0.09	0.34 \pm 0.04	0.20 \pm 0.05
Intestine	0.99 \pm 0.16	0.39 \pm 0.05*	0.93 \pm 0.06	0.87 \pm 0.03
Liver	0.60 \pm 0.14	0.62 \pm 0.07	0.22 \pm 0.04	0.17 \pm 0.02
Lung	0.88 \pm 0.20	0.49 \pm 0.06	0.30 \pm 0.06	0.27 \pm 0.03
Heart	0.45 \pm 0.11	0.39 \pm 0.04	0.09 \pm 0.02	0.07 \pm 0.01
Muscle	0.25 \pm 0.09	0.20 \pm 0.03	0.05 \pm 0.02	0.04 \pm 0.01

**P* < 0.05, *t* test.

Biodistribution

Results from biodistribution studies using the labeled radiopharmaceutical kit are presented in Table 1 as the percentage of injected dose per gram of tissue (% ID/g). Radiopeptide displayed rapid blood clearance with 0.40 \pm 0.11% ID/g at 1 hour. Fast clearance from the neurotensin receptor-negative tissues except the kidneys was found as well. Labeled peptide shows high uptake values in the neurotensin peptide receptor-positive organs. Intestine uptake was significantly decreased (0.99% ID/g vs. 0.39% ID/g at 1 h), by blocking the receptor through prior injection of cold peptide which confirms the specificity of radioconjugate. On the other hand, the uptake reduction in non-targeted tissues due to blocking dose was not significant. Scintigraphy study showed early uptake 4 h post injection of radiopeptide in kidney and bladder (Figure 4).

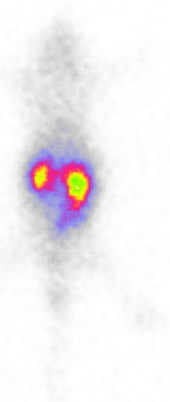


Fig 4. Scintigraphy image of radiolabeled kit in mice at 4 h post injection of 20 MBq of high specific activity radiotracer in 0.1 ml of saline via a tail vein.

DISCUSSION

Peptide receptors are overexpressed on various cancer cells and define interesting targets for tumour imaging and therapy. NT receptors were overexpressed in a variety of human tumors such as breast, pancreatic, prostate, lung and colon. Based on this fact and the experience with other peptides like somatostatin [13, 14] and Bombesin [15-18] it is reasonable to conclude that targeting the NT receptor with optimized analogue of neurotensin is very important for imaging of pancreatic, lung and colon tumors. In the present study, we investigated a new HYNIC coupled analog with substitution of Lys⁸ instead of Arg⁸ and D-Tyr¹¹ instead of Tyr¹¹ in neurotensin (8-13) sequences which lead to a substantial increase in metabolic stability. We attached HYNIC chelator via a Gly spacer to the N-terminus of neurotensin (8-13) peptide which can be labeled with more desirable radionuclide such as ^{99m}Tc.

A variety of chelators have been used as bifunctional chelating agent (BFCA) in labeling proteins, peptides and other biologically active molecule with ^{99m}Tc [9-11, 14, 19-21]. Among these, HYNIC is the best candidate because labeling in high specific activity is possible followed by application of various coligands, which permit control of the hydrophilicity and pharmacokinetics of the labeled peptide [15]. In the group of different coligands, tricine gives the best radiolabeling efficiency. However, it has been reported that due to different bonding modalities of the hydrazine moiety of the HYNIC and the tricine coligand, with tricine as a coligand, ^{99m}Tc-complex was not stable, particularly in dilute solutions [22]. The coligand EDDA is also of particular interest because it is a potentially tetradentate ligand and is

expected to form a more symmetrical and stable complex with technetium when compared to tricine [22]. It has been shown that using both coligands together to produce ^{99m}Tc -HYNIC-peptide via a trans-metallation type of reaction, produces very good results [23]. In this study we used 20 μg of HYNIC-peptide with tricine and EDDA together as a coligand in amounts of 15 mg and 5 mg in final radiopharmaceutical kit formulation respectively. We obtained high radiochemical yield $>98\%$ with very low amount of ^{99m}Tc -pertechnetate (<0.6) ^{99m}Tc -radiocolloid (<0.2) and ^{99m}Tc -coligands (<1.0). In RP-HPLC analysis we observed a single major peak without any impurities due to isomeric forms of labeled conjugate. In comparison to those report regarding ^{99m}Tc /tricine-HYNIC complex instability [24], our radiopharmaceutical kit was stable up to a 24 h post labeling period in the room temperature. These high labeling yield and stability may be due to optimization of formulation namely amount of material and our exchange labeling method.

Specific internalization was observed in this formulation ($12.13 \pm 0.35\%$ up to 4 h) which was not unexpected since neurotensin sequence offers agonistic property to the compound. Previous studies in a series of ^{99m}Tc -labeled neurotensin derivatives (NT (8-13), NT-I, NT-II, NT-IV, NT-VI, NT-X, NT-VIII, NT-XI, NT-XII and NT-XIX) and also Demotensin 1-4 demonstrate internalization and receptor mediated trapping of labeled compounds [11, 25]. These studies show that the highest rate of internalization corresponds to ^{99m}Tc -NT-II with 25.1% specific cellular uptake at 4 h, followed by ^{99m}Tc -Demotensin 4 reaching a 90-95% internalization plateau within 30 min [25]. Fast and receptor specific internalization demonstrable by uptake in NT receptor blocked cells experiments is an indication of stable binding properties for the complex by this formulation.

In biodistribution studies, clearance from the blood circulation was fast with 0.12% ID/g at 4 h and the whole body clearance mainly occurred via the urinary system. Clearance from NT receptor negative tissues was also rapid except from the kidneys. Accumulation of radiopeptide in neurotensin receptor positive tissues like the intestines was observed. The uptake was significantly reduced from 0.99 % ID/g in the control group (unblocked) to 0.39 % ID/g in the blocked group, which was in accordance with the evidence that has been found regarding NT receptor expression in the intestine and gastrointestinal tract [26, 27]. Uptake in the remaining organs was similar in both groups (unblocked and blocked). It could be concluded that increased intestinal uptake was caused by specific interaction with NT and also high in vivo stability of the designed formulation.

The gastrointestinal accumulation of this radiotracer and its favorable pharmacokinetic behavior such as low tendency to accumulate in liver and its high kidney excretion due to low lipophilicity and high stability are the major advantages of this lyophilized peptide radiopharmaceutical kit.

CONCLUSION

In this study, we have shown development of a new lyophilized peptide radiopharmaceutical kit. The labeling was completed within a very short time in high specific activity. Furthermore, this formulation prepared by labeling of peptide, HYNIC and tricine/EDDA as a coligands demonstrated an excellent radiochemical stability even up to 24 h post labeling. Our new formulation had a specific cell binding and internalization followed by a good stability in human serum at 37 °C for at least 24 h and no significant impurities were detected by HPLC. The prepared radiopharmaceutical showed high accumulation in intestine as a positive NT receptor targeted tissue followed by excretion via the kidney. These favorable characteristics make our new designed labeled peptide formulation as a promising candidate for diagnosis of malignant tumors.

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REFERENCES

1. Faintuch BL, Teodoro R, Duatti A, Muramoto E, Faintuch S, Smith CJ. Radiolabeled bombesin analogs for prostate cancer diagnosis: preclinical studies. *Nucl Med Biol.* 2008 May;35(4):401-11.
2. Reubi JC, Mäcke HR, Krenning EP. Candidates for peptide receptor radiotherapy today and in the future. *J Nucl Med.* 2005 Jan;46 Suppl 1:67S-75S.
3. Morinville A, Martin S, Lavallée M, Vincent JP, Beaudet A, Mazella J. Internalization and trafficking of neurotensin via NTS3 receptors in HT29 cells. *Int J Biochem Cell Biol.* 2004 Nov;36(11):2153-68.
4. Kitabgi P. Targeting neurotensin receptors with agonists and antagonists for therapeutic purposes. *Curr Opin Drug Discov Devel.* 2002 Sep;5(5):764-76.
5. Vincent JP, Mazella J, Kitabgi P. Neurotensin and neurotensin receptors. *Trends Pharmacol Sci.* 1999 Jul;20(7):302-9.
6. Reubi JC, Waser B, Friess H, Büchler M, Laissue J. Neurotensin receptors: a new marker for human ductal pancreatic adenocarcinoma. *Gut.* 1998 Apr;42(4):546-50.
7. Oyen WJ, Bodei L, Giammarile F, Maecke HR, Tennvall J, Luster M, Brans B. Targeted therapy in

- nuclear medicine--current status and future prospects. *Ann Oncol.* 2007 Nov;18(11):1782-92.
8. Schubiger PA, Allemann-Tannahill L, Egli A, Schibli R, Alberto R, Carrel-Rémy N, Willmann M, Bläuenstein P, Tourwé D. Catabolism of neurotensins. Catabolism of neurotensins. Implications for the design of radiolabeling strategies of peptides. *Q J Nucl Med.* 1999 Jun;43(2):155-8.
 9. Teodoro R, Faintuch BL, Núñez EG, Queiróz RG. Neurotensin(8-13) analogue: radiolabeling and biological evaluation using different chelators. *Nucl Med Biol.* 2011 Jan;38(1):113-20.
 10. Maina T, Nikolopoulou A, Stathopoulou E, Galanis AS, Cordopatis P, Nock BA. [^{99m}Tc]Demotensin 5 and 6 in the NTS1-R-targeted imaging of tumours: synthesis and preclinical results. *Eur J Nucl Med Mol Imaging.* 2007 Nov;34(11):1804-14.
 11. García-Garayoa E, Maes V, Bläuenstein P, Blanc A, Hohn A, Tourwé D, Schubiger PA. Double-stabilized neurotensin analogues as potential radiopharmaceuticals for NTR-positive tumors. *Nucl Med Biol.* 2006 May;33(4):495-503.
 12. Abrams MJ, Juweid M, tenKate CI, Schwartz DA, Hauser MM, Gaul FE, Fucello AJ, Rubin RH, Strauss HW, Fischman AJ. Technetium-99m-human polyclonal IgG radiolabeled via the hydrazino nicotinamide derivative for imaging focal sites of infection in rats. *J Nucl Med.* 1990 Dec;31(12):2022-8.
 13. Gandomkar M, Najafi R, Shafiei M, Mazidi M, Ebrahimi SE. Preclinical evaluation of [^{99m}Tc /EDDA/tricine/HYNIC(0, 1-NaI3, Thr8)]-octreotide as a new analogue in the detection of somatostatin-receptor-positive tumors. *Nucl Med Biol.* 2007 Aug;34(6):651-7.
 14. Erfani M, Shafiei M, Mazidi M, Goudarzi M. Preparation and biological evaluation of [^{99m}Tc /EDDA/Tricine/HYNIC(0), BzThi(3)]-octreotide for somatostatin receptor-positive tumor imaging. *Cancer Biother Radiopharm.* 2013 Apr;28(3):240-7.
 15. Sadeghzadeh N, Gandomkar M, Najafi R, Shafiei M, Sadat Ebrahimi SE, Shafiee A, Larijani B. Preparation and evaluation of a new ^{99m}Tc labeled bombesin derivative for tumor imaging. *J Radioanal Nucl Chem.* 2010;283:181-87.
 16. Sadeghzadeh N, Gandomkar M, Shafiee M, Mazidi M, Goudarzi M, Mirfallah SM, Sadat Ebrahimi SE. Synthesis and evaluation of a new radiolabeled bombesin analogue for diagnosis of GRP receptor expressing tumors. *Iran J Nucl Med.* 2009;17(1):18-26.
 17. Shirmardi SP, Gandomkar M, Mazidi M, Shafiei M, Ghannadi Maragheh M. Synthesis and evaluation of a new bombesin analog labeled with ^{99m}Tc as a GRP receptor imaging agent. *J Radioanal Nucl Chem.* 2011;288:327-35.
 18. Shirmardi SP, Gandomkar M, Maragheh MG, Shamsaei M. Preclinical evaluation of a new bombesin analog for imaging of gastrin-releasing peptide receptors. *Cancer Biother Radiopharm.* 2011 Jun;26(3):309-16.
 19. Liu S, Edwards DS, Looby RJ, Poirier MJ, Rajopadhye M, Bourque JP, Carroll TR. Labeling cyclic glycoprotein IIb/IIIa receptor antagonists with ^{99m}Tc by the preformed chelate approach: effects of chelators on properties of [^{99m}Tc]chelator-peptide conjugates. *Bioconjug Chem.* 1996 Mar-Apr;7(2):196-202.
 20. Kasina S, Rao TN, Srinivasan A, Sanderson JA, Fitzner JN, Reno JM, Beaumier PL, Fritzbeg AR. Development and biologic evaluation of a kit for preformed chelate technetium-99m radiolabeling of an antibody Fab fragment using a diamide dimercaptide chelating agent. *J Nucl Med.* 1991 Jul;32(7):1445-51.
 21. Maina T, Stolz B, Albert R, Bruns C, Koch P, Mäcke H. Synthesis, radiochemistry and biological evaluation of a new somatostatin analogue (SDZ 219-387) labelled with technetium-99m. *Eur J Nucl Med.* 1994 May;21(5):437-44.
 22. Liu S, Edwards DS, Looby RJ, Harris AR, Poirier MJ, Barrett JA, Heminway SJ, Carroll TR. Labeling a hydrazino nicotinamide-modified cyclic IIb/IIIa receptor antagonist with ^{99m}Tc using aminocarboxylates as coligands. *Bioconjug Chem.* 1996 Jan-Feb;7(1):63-71.
 23. Gabriel M, Froehlich F, Decristoforo C, Ensinger C, Donnemiller E, von Guggenberg E, Heute D, Moncayo R. ^{99m}Tc -EDDA/HYNIC-TOC and (^{131}I) whole-body scans. *Eur J Nucl Med Mol Imaging.* 2004 Mar;31(3):330-41.
 24. Babich JW, Fischman AJ. Effect of "co-ligand" on the biodistribution of ^{99m}Tc -labeled hydrazino nicotinic acid derivatized chemotactic peptides. *Nucl Med Biol.* 1995 Jan;22(1):25-30.
 25. Nock BA, Nikolopoulou A, Reubi JC, Maes V, Conrath P, Tourwé D, Maina T. Toward stable N4-modified neurotensins for NTS1-receptor-targeted tumor imaging with ^{99m}Tc . *J Med Chem.* 2006 Jul 27;49(15):4767-76.
 26. Tanaka K, Masu M, Nakanishi S. Structure and functional expression of the cloned rat neurotensin receptor. *Neuron.* 1990 Jun;4(6):847-54.
 27. Mulè F, Serio R, Postorino A, Vetri T, Bonvissuto F. Antagonism by SR 48692 of mechanical responses to neurotensin in rat intestine. *Br J Pharmacol.* 1996 Feb;117(3):488-492.