Novel indium-111 labeled gastrin peptide analogues (MG-CL1-4): Synthesis and quality control

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Abstract: Radiolabeled neuropeptides are widely investigated to diagnose and therapy of tumors. These peptides get internalization after binding with particular receptors at the surface of cells and finally move to lysosome. Internalization into tumor cells helps in mapping the infected site. Minigastrin peptide analogues (MG-CL1-4) were synthesised and labeled with 111-In radioisotope under different sets of conditions for imaging CCk-2 receptor bearing tumors. Different parameters such as temperature (80-100°C), pH (4-12), incubation time (5-30 minutes) and dilution effect were investigated to get the maximum labeling yield and stability. The results indicated that MG-CL1-4 is successfully labeled with indium-111 at pH 4.5 with heating at 98°C for 15 minute. At these conditions i.e. heating, pH and incubation minimum oxidized and maximum labeling yield, more than 94 %, was obtained. The labeling stability was studied by incubating the radiolabeled complex for predefined time points in PBSA and blood serum. Results show that more than 90% radiolabeled MG-CL1-4 remained intact.

Keywords: Indium-111, minigastrin peptides, MG-CL, labeling and gastrin hormone

INTRODUCTION

The development of radiolabeled neuropeptides as a tool to target malignant tumors for either imaging or radiotherapeutic purposes is focus of interest in nuclear medicine research (Mather *et al.,* 2007; Reubi *et al.,* 1997; Sosabowski *et al.,* 2007). Several receptor systems, for example, Somatostatin, VIP, CCk-B, CCk-A, Substance-P, Bombesin/GRP, Neurotensin etc., (Behr *et al.,* 1999; Mather 2007; Naqvi *et al.,* 2010) have been investigated for the development of peptide-based radiopharmaceuticals for imaging and treatment (Cornelio *et al.,* 2007). Following the binding of neuropeptides to their respective receptors on the tumor cell surface, internalization into intracellular vesicles occurs as a result of endocytosis that makes the bases of imaging (Aylin *et al.,* 2008).

The unstable radioligand degrades before reaching the surface of tumor cells and subsequently into the lysosome. This behaviour causes different levels of radiotoxicity in blood stream, body tissues and in different organs due to non specific accumulation. Particularly, the unstable radioconjugates on moving toward tumor cells through blood stream may degrade due to its physiochemical conditions and by the action of enzymes present in the blood. Presence of stability correlation between *in vitro* and *in vivo* conditions leads to establish successful radio-pharmaceuticals (Ocak *et al.,* 2011). Duncan *et al* (1987) introduced enzyme substrate sequence into the sequence of peptide in order to enhance the stability in the blood and release of radiopeptides into the cytoplasm (Duncan 1987). The target to non target ratio depends on the labeling stability against physiochemical and enzymatic action of blood.

The gastrin peptide sequence -EAYGWMDF-NH_2 is generally felt to be the prerequisite requirement for high binding affinity for the receptors (Mather *et al.,* 2007). We designed and synthesis the series of four minigastrin peptides having a cathepsin-labile site to reduce unexpected radioconjugate degradation. We introduced a cathepsin B proteolytic site, GFLG, (Lu *et al.,* 2002) and then di-His after receptor-binding sequences, and, in order to generate a variety of different radiometabolites with, presumably different properties, subsequently substituted other amino acids for the histidine residues. The full list of peptides studied is shown in table 1. A DOTA chelator introduced for radiolabeling with 111-In and a dihistidine

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pharmacokinetic modifying sequence, which reduces the renal accumulation of the peptide. Prior to *in vivo* study of radioconjugates, it is necessary to optimize the conditions for stable radiolabeling and proper stability in blood serum (Naqvi *et al.,* 2012; Di Pierro *et al.,* 2008; Gandomkar *et al.,* 2008).

In this study, novel minigastrin peptides were synthesised and labeled with indium-111. The labeling conditions were studied and optimized to minimise the formation of oxidised radioconjugate product and maximize the labeling yield. Finally the stability of radiolabeled MG-CL1-4 peptides was tested using fresh human blood plasma.

MATERIALS AND METHODS

Chemicals

MGCL1-4 were synthesized by solid-phase 9-flourenylmethyloxycarbonyl (Fmoc) chemistry with >95% purity in Cancer Research, UK. All chemicals were of analytical grade and purchased from Fisher Scientific (Loughborough, UK), with the following exceptions: acetonitrile (ACN), triisopropylsilane (TIS) and trifluoroacetic acid (TFA) for high-performance liquid chromatography (HPLC) grade; gentisic acid, *N,N*-diisopropylethylamine (DIEA), dimethylformamide (DMF), N-Methylpyrrolidone (NMP), trypan blue, and ethylene diamine tetraacetic acid (EDTA) were purchased from Sigma-Aldrich (Gillingham, UK), and hydrochloric acid was purchased from VWR (East Grinstead, UK). $^{111}InCl_3$ in 0.05M HCl was purchased from Covidien (Petten, Netherlands).

Synthesis of minigastrin peptides

The minigastrin peptides were synthesised with the help of automated multiple peptide synthesizer MultiPep (Intavis AG, Germany) using standard 9-flourenylmethyloxycarbonyl (Fmoc) solid phase peptide synthesis (SPPS) strategy. Rink Amide MBHA resin was used as solid support. When the sequence each of the four gastrin peptide analogues was completed, the Fmoc N-terminal protecting group was removed and the peptide resin was transferred to a manual vessel for coupling of the 1,4,7,10-tetraazacyclodecane-N,N',N'',N'''-tetra-acetic acid (DOTA).

The DOTA was coupled to histidine or aspartic acid or leucine or aspergine amino acid. For DOTA conjugation, the peptide-resin beads were then pre-swollen with 200 µl on NMP. A mass of 115 µg (0.2 mmols, 4 fold excess) of DOTA was placed in a microfuge tube followed by the addition of 200 µl aliquot of 1 M HOBT in NMP. After adding 6.5 fold excess of N, N/-diisopropylcarbodiimide (DIC) to the DOTA solution, the solution was incubated at room temperature for 30 minutes to activate. The "Activated DOTA" was added to the swollen resin. The tube was sealed and placed on a rotator for 24 hours to couple. The resin beads was filtered and washed with at least 3 washes of NMP. The Ninhydrin test was carried out on the beads, where a complete reaction produced greyish beads and pale yellow solution and purple solution indicated a partial reaction or no reaction occurred. Then the DOTA addition procedure was repeated.

The cleavage and deprotection was performed by treating the fully protected DOTA-peptide resin with TFA (92.5%) containing water (2.5%), TIS (2.5%) and EDT (2.5%). The DOTA coupled peptides (MGCL 1-4) were precipitated with 1 mL of diethyl ether cooled on ice. Triple washed (using ice cold ether) compounds were freeze dried from a 2% acetic acid/water solution. The resultant solids were then purified by reverse-phased (RP) high pressure liquid chromatography (HPLC) with a C_8 column; elution was done by the use of solvent A (H_2O:0.1% TFA) and solvent B (CH_3CN:0.1% TFA) linear gradients with 5%-95% B over 45 minutes at flow rate of 1 mL/min. Purified samples were precipitated with ice cold ether and freeze dried at average temperature -77 °C and 0.02 mbar vacuum. For purity confirmation HPLC analysis and mass spectrometry was done.

Radiolabeling

Labeling was performed in 1.8mL screw-top polypropylene vials. Labeling conditions of novel minigastrin analogues were tested by different parameters to obtain stability of product and maximum labeling yield. Labeling conditions were adjusted by studying pH, concentration of MGCL peptide and radioisotope, buffer system, temperature and time required for reaction. MGCL labeling with In-111 was tested for a range of ligand concentration 0.25 mg to 2.0 mg/mL, moderate acidic to basic pH (3-12). Temperature effect was studied by changing from room temperature to 100 ºC, reaction medium was studied using water, PBSA and phosphate buffer system, after each labeling study the reaction was quenched with the addition of 1.8 µL of 0.1 M EDTA solution.

Instant thin layer chromatography (ITLC)

ITLC was performed on silica gel ITLC Strips (ITLCTM SG, Gelman Sciences) using different mobile phases. First mobile phase, 50 mM EDTA in 0.1 M sodium acetate and 3.5% v/v ammonia/water: methanol 1:1, were used for ^{111}In-MG-CL (Rf=0) and ^{111}In-EDTA (Rf=1) and second mobile phase, ammonia/ water: methanol, was used for both ^{111}In-MG-CL and ^{111}In-EDTA eluted to the solvent front (Rf=1) while any insoluble material or colloid formation remained at the origin (Rf=0). The strips were imaged on a storage phosphor imaging system (CycloneTM, Packard). The images were then quantified using compatible analysis software (Optiquant v.3.00, Packard).

Reverse-phase high performance liquid chromatography (RP-HPLC)

The radiochemical purity of labeled peptides was determined by reverse phase high performance liquid chromatography (RP-HPLC), using Beckman HPLC systems, consisting of Beckman 125 or 126 solvent-module pump systems with a Beckman 166-NM single wavelength or 168 diode array ultraviolet (UV) detectors (Beckman, High Wycombe, England) and a sodium iodide flow-through radiochemical detector attached to a Raytest gamma-radioactivity monitor. Two different mobile phases were used for RP-HPLC analysis, one is 0.01M TEAA/UHP water (A) and 100% acetonitrile (ACN) (B) and second is 0.1% trifluoroacetic acid (TFA)/water (A) and acetonitrile (B), at a flow rate of 1mL/min was used. The gradient was performed as follows: 0 min 95% A (5% B), 5 min 95% A (5% B), 20 min 40% A (60% B), 25 min 0% A (100% B), 29 min 95% A (5% B).

Blood plasma stability assay

For radiolabeled peptides stability studies, the radiolabeling was performed according to the protocol and diluted the radioligand using either of the solvent, water or PBS. The pH of the labeled product was set from 7-12 with 0.1M NaOH and noted the effect on the stability of complex against predefined time points. For plasma stability, samples of ^{111}In-MG-CL(1-4) were incubated at 37 $^{\circ}$C in PBS solution and freshly harvested human blood plasma at 0, 1, 2, 4 and 24h time points. Similar study was performed with ^{111}In-APH-O70 as control. Ice cold acetonitrile (ACN) and aliquot were added in a 3:1 (v/v) ratio. After centrifugation, supernatant samples were analyzed by RP-HPLC and ITLC.

RESULTS

Minigastrin peptides, MG-CL1-4, were synthesised by standard solid phase peptide synthesis, DOTA was coupled off-line to the N-terminal of the resin bound peptides. This coupling step was long enough of 24 hours, however resin cleavage and deprotection was done in two hours to make sure complete removal of *t*-Butyl and other protecting groups. Agilent 1100 series mass spectrometer equipped with RP-HPLC was used to verify the molecular mass and purity of the peptides. The results showed high purity and yield (>32%) in all cases. For example the Mass spectrum analysis of MG-CL1 as shown in fig. 1, express excellent ion peak at 2050.91 amu, indicates the high purity which is very near to calculated mass that is 2052.84 amu. Similarly all other peptides have shown similar level of purity as shown in table 1.

MG-CL1-4 peptides were labeled with 111-In using different set of conditions. The labeling yield was found highly dependent on pH (table 2), incubation time and temperature. Methionine present at third position from c-terminal of minigastrin peptide is heat sensitive in its oxidation (Sosabowski *et al.,* 2007). Oxidation, particularly of methionin residues, is one of the major chemical denaturing of proteins (Mulinacci *et al.,* 2011). Oxidation of methionine in a peptide sequence leads to reduce the receptor binding affinity (Mather *et al.,* 2007). A study was performed to select appropriate value of pH, incubation time and temperature for least methionine oxidation and maximum labeling yield. Initially labeling was performed at pH 4.5 using a temperature range 80-100°C for different incubation period (5-30 minutes). Appropriate yield of nonoxidized radioconjugate (>85%) was obtained at 96-100°C temperature and 15 minutes incubation period as shown in the fig. 2. The temperature range from 90-100°C was observed more suitable for labeling the minigastrin analogues. In short, heating at 98°C for 15 minutes at pH 4.5, produced a maximum nonoxidized radioconjugate product and the results were also found reproducible.

Table 2: The effect of pH on the stability of 111-In-MG-CL1

pH	Percent stability when diluted with	
	PBS	PO_4^- Buffer
7.5	≈100	≈97
8.0	≈100	95.67
8.5	95.98	90.11
9.0	94.45	84.91
9.5	89.02	80.47
10.0	82.98	79.06
10.5	83.48	70.13
11.0	76.73	68.21
12	67.41	63.05

Table 1: Mass spectrometric and RP-HPLC analysis of minigastrin peptides (MG-CL1-4)

Sr. No.	Peptide	Nomenclature	Mass spectrum		HPLC	
			m/z (peptide ion)	Calculated Mass	T_R (min)	Purity (%)
1	DOTA-HH-GFLG-EAYGWMDF-NH2	MGCL-1	2050.91	2052.84	6.93	96.78
2	DOTA-DD-GFLG-EAYGWMDF-NH2	MGCL-2	2006.89	2008.72	7.49	95.64
3	DOTA-LL-GFLG-EAYGWMDF-NH2	MGCL-3	2003.07	2004.86	8.53	97.24
4	DOTA-NS-GFLG-EAYGWMDF-NH2	MGCL-4	1978.00	1979.72	7.41	98.11

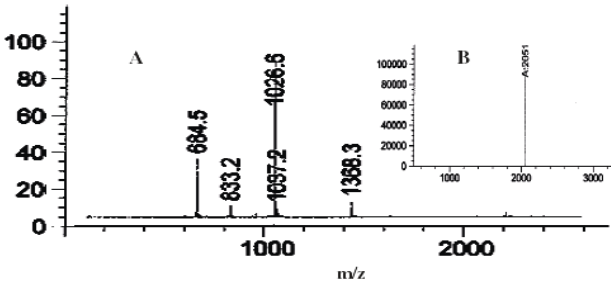

Fig. 1: Mass spectrum of MG-CL1

However, higher pH was found non satisfactory for labeling process. The stability of the radioconjugate complex was studied at different pH (7-12) after dilution with any of three solvents, DI water, PBS or phosphate buffer. 111-In-MG-CL1 complex was quite stable up to pH 9 in case of dilution with DI water or PBSA. Above this pH continuous decrease in stability was seen and only 67.41% complex was left at pH 12. While dilution with PO_4^- buffer was not feasible, in this medium the percent stability of the radioligand was 84.91% at pH 9 which was decreased to 63.05% at pH 12. The solvent/buffer chosen for dilution after labeling proved to be important. Phosphate buffer interfered with the stability of the radiolabeled peptide. Dilution with DI water or PBS maintains the stability of the complex (table 3). The labeling performed at 98°C, 15 minutes incubation and pH 4.5 found to be more stable than any other condition.

Table 3: ^{111}In-MG-CL1 stability upon dilution with water, PBS or Phosphate (PO_4^-) buffer.

Time (minutes)	Percent of Radioligand		
	Water	PBS	PO_4^- Buffer
0	100	100	100
30	100	100	97
60	99	100	96
120	99	99	95

Finally labeling was performed in 1.8mL screw-top polypropylene vials by the consecutive addition of 20 mL of $^{111}InCl_3$, 4 mL 1M ammonium acetate (pH 5.5) containing 8.3mg/mL gentistic acid and 4–5 mL of a

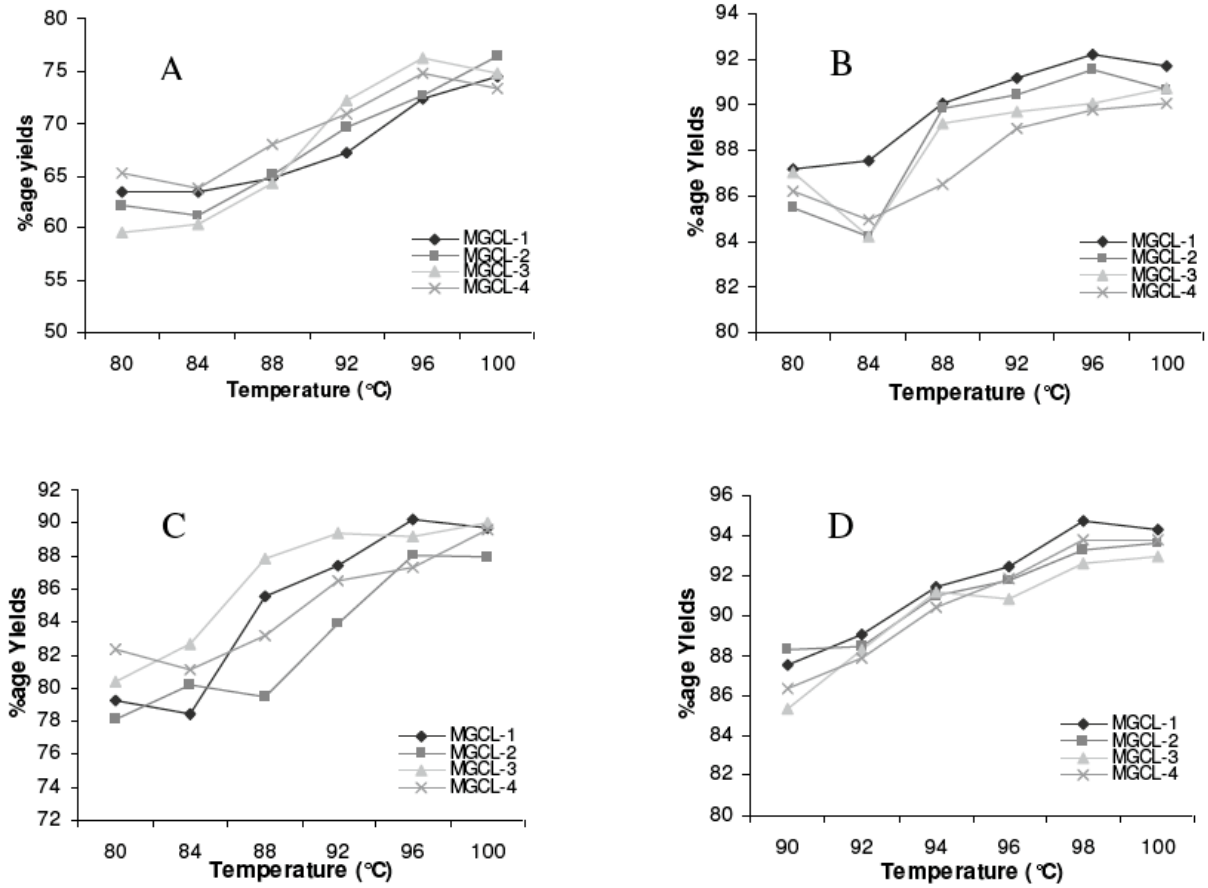

Fig. 2: Effect of temperature and incubation time at pH 4.5 on labeling yields of MGCL1-4; A) Incubation for 10 minutes and 80-100°C temperature range; B) Incubation for 15 minutes and 80-100°C temperature range; C) Incubation for 30 minutes and 80-100°C temperature range; D) Incubation for 15 minutes and 90-100°C temperature range.

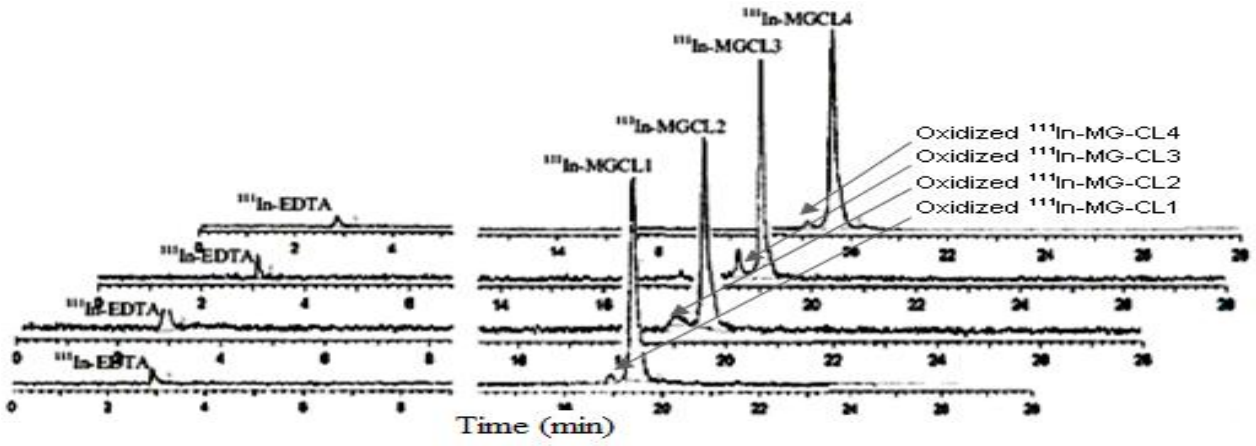

Fig. 3: RP-HPLC analysis showing the retention time of 111-In-MG-CL1-4 peptides (19.5 min, 20 min, 19 min and 20 min respectively). The main peaks at its base contain a small fraction of oxidized radiolabeled MG-CL1-4.

1mg/mL aqueous solution of MG-CL1-4. The mixture as incubated for 15 minutes at 98°C in a dry heating block, after which the reaction was quenched by the addition of 1.8 mL of 0.1 M EDTA solution. Then dilution was performed either with deionised water or PBSA solution for *in vitro* study.

The nonoxidized radioconjugate thus obtained under these conditions were >85%, as assessed by RP-HPLC which indicates minute fraction (<4%) of ^{111}In-EDTA elution along solvent front while a small shoulder of oxidized radioconjugate (≤4%) were appeared at the base of non-oxidized radioconjugate (≥85%) as shown in the fig. 3. Anyhow overall yield analysed by ITLC were found more than 94% as shown in the strip A, only a minute fraction of ^{111}In-EDTA was eluted at solvent front while in strip B no traces of colloid were found at the origin of the strip (fig. 4).

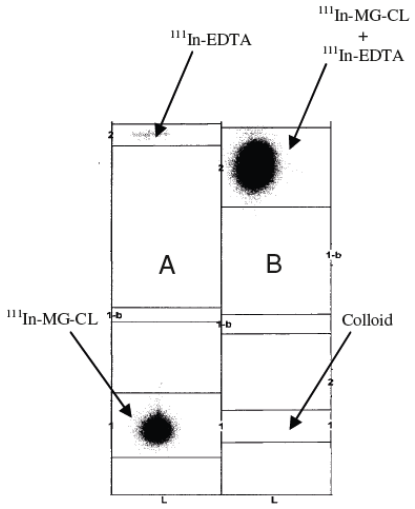

Fig. 4: ITLC analysis; A) minute fraction of 111-In-EDTA complex formation which eluted at solvent front. B) No traces of colloid were found at the origin of the strip.

Peptide radiopharmaceuticals stability in blood plasma has a key role for accumulation of radiopharmaceuticals in tumour (Good *et al.*, 2008). Blood plasma stability assay revealed no significant differences in stability among all the MG-CL1-4 peptides in human serum. The results show more than 90% of peptides remain intact after 2 hours of incubation in human serum (fig. 5).

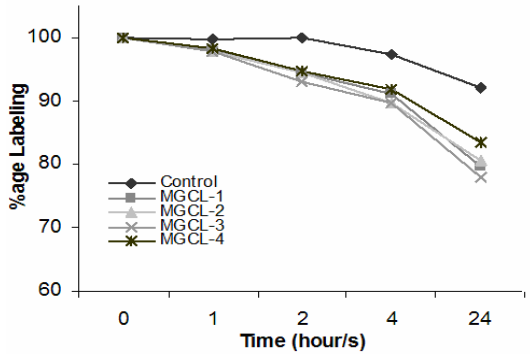

Fig. 5: Plasma stability studies of 111-In-MG-CL1-4 radiopeptides

DISCUSSION

Radiolabeled peptides have gained serious attention for both diagnosis and therapy of death leading infections and cancers. Peptides which are synthesised on the bases of biomolecules (hormones), most commonly work through receptor binding. The purity and adequate receptor sensing ability of the peptides are prerequisite for radiolabeled peptide therapy (RPT). Solid phase synthesis of gastrin hormone peptide analogues (MG-CL1-4) satisfies purity of the analogues while quality control of the radiolabeled peptides assures the receptor binding ability. More than 95% purity was obtained in each case of minigastrin peptide synthesis. The success of the diagnosis and therapeutic strategy mainly depends on

radiolabeling yield, purity, stability, non-oxidative product if methionine present in peptide sequence and accumulation in the diseased cells. Our results show very impressive radiolabeling yield, purity and stability of radioconjugates.

Radiolabeling of these peptides with 111-In was performed using a set of parameters i.e. pH, incubation time and temperature. Temperature was the most considerable parameter during the radiolabeling process because the receptor binding sequence –Trp-Met-Asp-Phe-NH_2 with CCK-2 receptor was more sensitive to methionine oxidation. Methionin present in receptor binding peptide sequence additionally posses the risk of methionine oxidation, especially during heating step of radiolabeling process (von Guggenberg *et al.,* 2007). Cell internalization rate decreases drastically when ratio of oxidative side product increases. This shows the decrease in affinity of oxidative product for CCK-2 receptors. Reaction conditions are very important to minimize the methionine oxidation and in turn to increase the tumor uptake. Breeman et al., have reported that adjustment of reaction conditions can minimize the oxidation of methionine (Breeman *et al.,* 2006). Our results show that the reaction conditions, 15 minutes incubation at 98°C and 4.5 pH give least oxidative product (<5%).

Stability of the radiolabeled peptides have direct impact on tumor uptake of radiopharmaceuticals. The stability of the radioconjugate was studied in different dilution buffers and at different pH values as shown in table 2. The results of this study indicated appreciable stability in both PBS and PO_4^- buffer. The similar stability was also appeared when the radioconjugates were diluted with de-ionized water. Good *et al*., have indicated the role of radioconjugate stability in blood stream while travelling toward target tissues (Good *et al.,* 2008). Less stable radioconjugate molecules while travelling through the blood stream become fragmented by enzymatic action. These fragmented radioconjugates no longer would be able to reach at tumor cells but can impose serious radiotoxicity. The Blood plasma stability assay of 111-In-MG-CL1-4 has shown impressive results. Figure 5 indicates the stability (>90%) of all 111-In-MG-CL peptides during first 4h. Very promising stability may be the indication of maximum tumor uptake of receptor positive radiolabeled minigastrin peptides.

CONCLUSION

Metabolically stable radiopharmaceuticals guarantied the successful diagnosis and therapy of malignant tissues. It is necessary to investigate in vitro stability of radioconjugates prior to in vivo study. A series of systematic experiments were performed to choose stable radiolabeling protocol. The set of radiolabeling conditions that were adjusted, gave excellent labeling and metabolic stability. The results obtained from this study might be used to study these radiolabeled peptides in vivo system. The study of these radiolabeled peptides in living system has been published before this publication due to promising results. However, due to strong stability correlation between in vitro and in vivo results these radiolabeled compounds may be tested clinically.

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REFERENCES

Aylin C, Hanyaloglu and Zastrow M (2008). Regulation of GPCRs by Endocytic membrane trafficking and its potential implimentations. *Annu. Rev. Pharmacol. Toxicol.,* **48**: 537-568.

Behr TM, Behe M and Becker W (1999). Diagnostic applications of radiolabeled Peptides in nuclear endocrinology. *QJ. Nucl. Med.,* **4***:* 268-280.

Breeman WAP, de Blois E, van Gameren A, Melis M, Fröberg A, de Jong M, *et al.* (2006). Aspects of CCK-2 Receptor-Targeting with 111In-DOTA-MG. *In*: Mazzi U, editor. Technetium, rhenium and other materials in chemistry and nuclear medicine 7. Padova: SG Editoriali, pp. 231-232.

Cornelio DB, Roesler R and Schwartsmann G (2007). Gastrin-releasing peptide receptor as a molecular target in experimental anticancer therapy. *Ann. Oncol.,* **18**: 1457-1466

Di Pierro D, Rizzello A, Cicoria G, Lodi F, Marengo M, Pancaldi D, Trespid S and Boschi S (2008). Radiolabelling, quality control and radiochemical purity assessment of the Octreotide analogue ^{68}Ga DOTA NOC. *App. Radi. Isotop*., **66**: 1091-1096.

Duncan R (1987). Anticancer agents coupled to N-(2-hydroxypropyl) methacrylamide copolymers. I. Evaluation of daunomycin and puromycin conjugates *in vitro. Br. J. Cancer.,* **55**: 165-174

Gandomkar M, Najafi R, Mazidi M, Goudarzi M and Ebrahimi SES (2008). Synthesis, labeling and quality control of a new neuropeptide Y analogue for diagnosis of breast tumors. *Iran J. Nucl. Med.,* **16**: 20-24.

Good S, Walter MA, Waser B, Wang X, Muller-Brand J, Behe MP, Reubi JC and Maecke HR (2008). Macrocyclic chelator-coupled gastrin-based radio-pharmaceuticals for targeting of gastrin receptor-expressing tumour *Eur. J. Nucl. Med. Mol. Imag.,* **35**: 1868-1877.

Lu ZR, Shiah JG, Sakuma S, Kopecková P and Kopecek J (2002). Design of novel bioconjugates for targeted drug delivery. *J. Contr. Rel.,* **78**: 165-173.

Mather SJ, McKenzie AJ, Sosabowski JK, Morris TM, Ellison D and Watson SA (2007). Selection of radiolabeled gastrin analogs for peptide receptor-targeted radionuclide therapy. *J. Nucl. Med.,* **48**: 615-622.

Mather SJ (2007). Design of radiolabelled ligands for the imaging and treatment of cancer. *Mol. Biosyst.,* **3**: 30-35.

Mulinacci F, Capelle MAH, Gurny R, Drake AF and Arvinte T (2011). Stability of human growth hormone: Influence of methionine oxidation on thermal folding. *J. Pharm. Sci.,* **100**: 451-463.

Naqvi SAR, Matzow T, Finucane C, Nagra SA, Ishfaq MM, Mather SJ and Sosabowski J (2010). Insertion of a lysosomal enzyme cleavage site into the sequence of a radiolabeled neuropeptide influences cell trafficking in vitro and in vivo. *J. Cancer* Biother. *Radiopharm.,* **25**: 89-95.

Naqvi SAR, Ishfaq MM, Khan ZA, Nagra SA, Bukhari IH, Hussain AI, Mahmood N, Shahzad SA, Haque A and Bokhari TH (2012). 99*m*Tc labelled levofloxacin as an infection imaging agent: a novel method for labelling levofloxacin using cysteine·HCl as co-ligand and in vivo study. *Turk. J. Chem.,* **36**: 267-277.

Ocak M, Helbok A, von Guggenberg E, Ozsoy Y, Kabasakal L, Kremser L and Decristoforo C (2011). Influence of biological assay conditions on stability assessment of radiometal-labelled peptides exemplified using a 177Lu-DOTA-minigastrin derivative, *Nucl. Med. Bio.,* **38**: 171-179.

Reubi JC (1997). Regulatory peptide receptors as molecular targets for cancer diagnosis and therapy. *QJ. Nucl. Med.,* **41**: 63-70.

Sosabowski JK, Lee M, Dekker BA, Simmons BP, Singh S, Beresford H, Hagan SA, McKenzie AJ, Mather SJ and Watson SA (2007). Formulation development and manufacturing of a gastrin/CCK-2 receptor targeting peptide as an intermediate drug product for a clinical imaging study. *Eur. J. Pharm. Sci.,* **31**: 102-111.

von Guggenberg E, Dietrich H, Skvortsova I, Gabriel M, Virgolini IJ and Decristoforo C (2007). 99mTc-labelled HYNIC-minigastrin with reduced kidney uptake for targeting of CCK-2 receptor-positive tumours. *Eur. J. Nucl. Med. Mol. Imaging,* **34**: 1209-1218.