STUDY OF THE RADIOLABELING OF SUBSTANCE P WITH LUTETIUM-177 AND ANALYSIS OF THE STABILITY *IN VITRO*: DEVELOPMENT OF NEW RADIOPHARMACEUTICAL FOR TUMOR TREATMENT

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ABSTRACT

Substance P (SP) is an 11- amino acid neuropeptide, which is known as an important member of the family of the tachykinins, characterized by the C-terminal sequence Phe-X-Gly-Leu-Met-NH_2. Radiolabeled SP has been described and proposal for detection and treatment of diseases such as arthritis and tumors. SP is the most important target of neurokinin 1 (NK-1) receptors, overexpressed in malignant gliomas. ^{177}Lu is commonly used in the production of radiopharmaceuticals for treatment of neuroendocrine tumors and is a radionuclide with favorable properties for endoradiotherapy. The half-life of ^{177}Lu is 6.75 days and it emits β- particles of 497 keV average energy. Moreover, ^{177}Lu also emits γ radiation of 208 keV average energy, which makes imaging diagnosis possible. There are few studies describing radiolabeled SP analogs in literature and the objective of this work was to study the radiolabeling conditions and the stability of SP complexed to DOTA chelator, using ^{177}Lu as radionuclide, in order to determine the best radiolabeling methodology. A high radiochemical purity (> 95%) and high specific activity of DOTA-SP was achieved when the reaction time was 30 minutes, the temperature was 90 ºC, the mass of DOTA-SP was 10 μg and ^{177}Lu activity was 185 MBq. These conditions extrapolate will be used in future experiments with high activity and also in *in vitro* and *in vivo* studies involving glioma models.
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1. INTRODUCTION

Substance P (SP) is an 11-amino acid neuropeptide which is known as a powerful member of a family of tachykinins characterized by the C-terminal sequence Phe-X-Gly-Leu-Met-NH_2. It has been well established that SP plays an important role in modulating pain transmission from peripheral and central primary afferents through neurokinin 1 and 2 receptors and this peptide may be also involved in the pathogenesis of inflammatory diseases [1]. SP receptors are also found in brain, lymphoid tissues, vessels, gut smooth muscle, airway glands and bronchiolar walls. In receptor autoradiography of tumor specimens *ex vivo*, SP receptors were found to be more abundant than somatostatin receptors on glioblastoma, medullary thyroid cancer (MTC), non-small cell lung cancer and carcinoma of pancreas, but the incidence is low in the latter two. In addition, SP receptors were also found on peritumoral vessels associated with those tumors [2].

In recent years, a number of new developments in targeted therapies have emerged [3] and the

presence of peptide receptors and transporters at the cell membrane of several NETs constitutes the basis of the clinical use of specific radiolabeled ligands. The introduction of radiolabeled SP analogs for peptide receptor imaging and radiotherapy can be a focus of interest to characterize and treat of tumors neuroendocrinos. Several radionuclides have been applied to label peptides for radionuclide therapy and 6.7 day half-life ^{177}Lu has emerged as a promising short-range β □emitter for this purpose. The mean range of lutetium-177 β particles ($E\beta_{max}$ = 497 keV) is 670μm, making this radionuclide ideal for treating micro-metastatic disease. Because it also emits γ rays (208keV, 11% abundance), imaging of ^{177}Lu-labeled endoradiotherapeutic agents is possible [4].

The goal of this work was to study the radiolabeling conditions and the stability of substance P complexed to DOTA chelator, using ^{177}Lu as radionuclide.

2. MATERIALS AND METHODS

2.1. Reagents

DOTA-Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2 (DOTA-Substance P - piChem) was provided from IAEA and $^{177}LuCl_3$ was obtained from NRG (Netherlands). All other chemicals and reagents required for experiments were of analytical grade and purchased from Sigma Aldrich Chemical Co..

2.2. Study of radiolabeling conditions

Substance P was radiolabeled at different conditions in order to determine the best radiolabeling methodology. The parameters studied were temperature (70 – 90 °C), peptide mass (0,5 - 10 μg), time of reaction (15 - 30 minutes) and $^{177}LuCl_3$ activity (37 - 400 MBq).

2.3. Radiochemical purity determination

Radiochemical purity was determined by instant thin layer chromatography (ITLC) was, with citrate/citric acid buffer pH 5.0 as solvent (Rf of labeled peptide was 0.1-0.3 and Rf of free lutetium was 0.9-1.0) [5]. Labeled Substance P was also analysed by HPLC (Shimadzu) using RP C_{18} columns (Waters, 4.0 x 150 mm, 5 μm) with radioactivity (Shell) detection, flow rate of 1.5 mL/minute with a linear gradient of 10-90% (v/v) 0.1% trifluoreacetic acid (TFA) / acetonitrile in 0.1% TFA / H_2O for 15 minutes and the composition was maintained for another 10 minutes.

2.4. Stability of radiolabeled SP

To determine the *in vitro* stability of ^{177}Lu-DOTA-SP the preparation was stored at refrigerator for different times (1 to 7 days) followed by ITLC analysis. All experiments were performed in duplicate.

2.5. Data analysis

Statistical analyses were performed using Prism 5.0 software. Results were subjected to Student´s *t*-test and expressed as mean ± SD.

3. RESULTS

3.1 Radiolabeling of SP with Lutetium-177

Different Substance P mass (0.5 – 10 μg) were radiolabeled using 92.5 MBq (2.5 mCi) of radionuclide and the results are shown in FIG. 1. High radiolabeling yields (> 95%) were achieved when 5 and 10 μg of DOTA-SP reacted with lutetium-177. When the mass of DOTA-SP was reduced to 2.5 μg the radiochemical purity decreased to 39.13 ± 3.2 and no radiolabeling reaction occurred using 1 and 0.5 μg of the peptide.

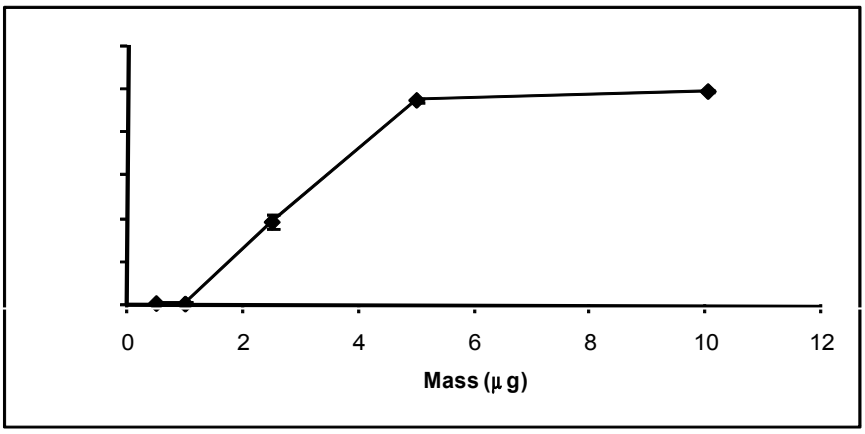

FIG. 1. Radiochemical purity of ^{177}Lu-DOTA-SP applying different ^{177}Lu activities (mCi). The reactions were performed at 90 °C for 30 minutes. At 10 μg of SP and 92.5 MBq of ^{177}Lu a radiochemical purity of 99.81 ± 0.1% was obtained.

The Tables I and II show the effects of the radiolabeling time and temperature on the radiochemical purity of labeled SP. The reactions were performed at 90 °C using 0.25 mCi/μg for different times. Labeling yield determined by ITLC was satisfactory in all times and temperature analysed.

Table I. Effect of the incubation time on radiochemical purity of ^{177}Lu-DOTA-SP.

	Radiolabeling time			
	15 minutes	20 minutes	25 minutes	30 minutes
Radiochemical Purity (%)	99.15 ± 0.8	98.17 ± 1.5	98.13 ± 0.1	99.81 ± 0.1

Table II. Effect of the temperature on radiochemical purity of ^{177}Lu-DOTA-SP.

	Radiolabeling temperature		
	70 °C	80 °C	90 °C
Radiochemical Purity (%)	95.33 ± 3.2	99.73 ± 0.1	99.81 ± 0.1

The FIG. 2 shows a typical radioactive HPLC profile of both lutetium-177 (A) and ^{177}Lu-DOTA-SP (B). The labeled peptide (Rt = 7.02 minutes) could be clearly separated from free lutetium (Rt = 1.15 minutes). No contamination by free lutetium was observed on ^{177}Lu-DOTA-SP HPLC profile.

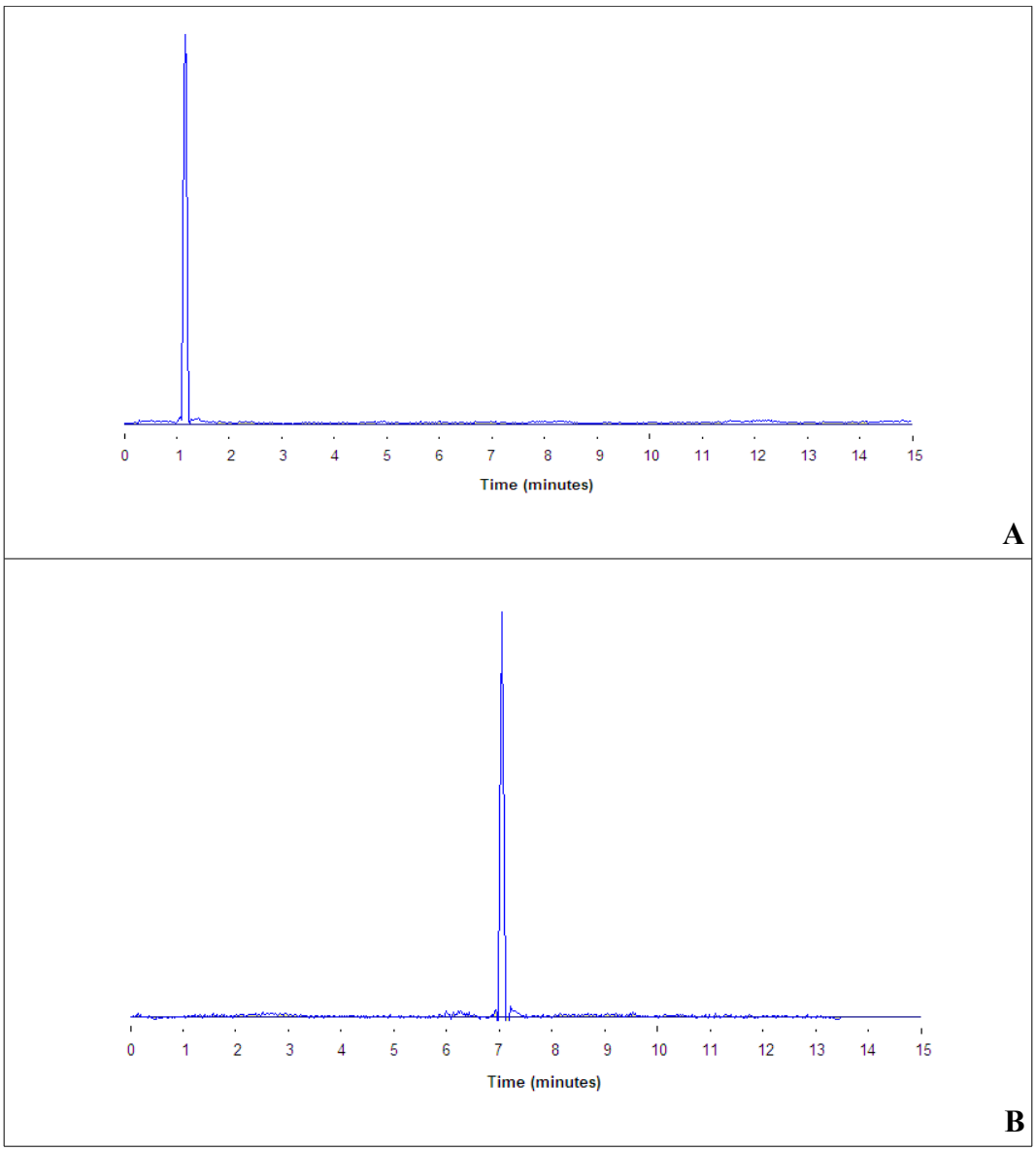

FIG. 2. HPLC profiles of (A) free lutetium-177 and (B) radiolabeled substance P.

The FIG. 3 shows the effect of the activity of ^{177}Lu on the radiochemical purity of the reactions. The mass of the SP was mainteined in 10 µg and ^{177}Lu activity varied from 37 to 370 MBq. The reaction was carried through 90 ºC in 30 minutes.

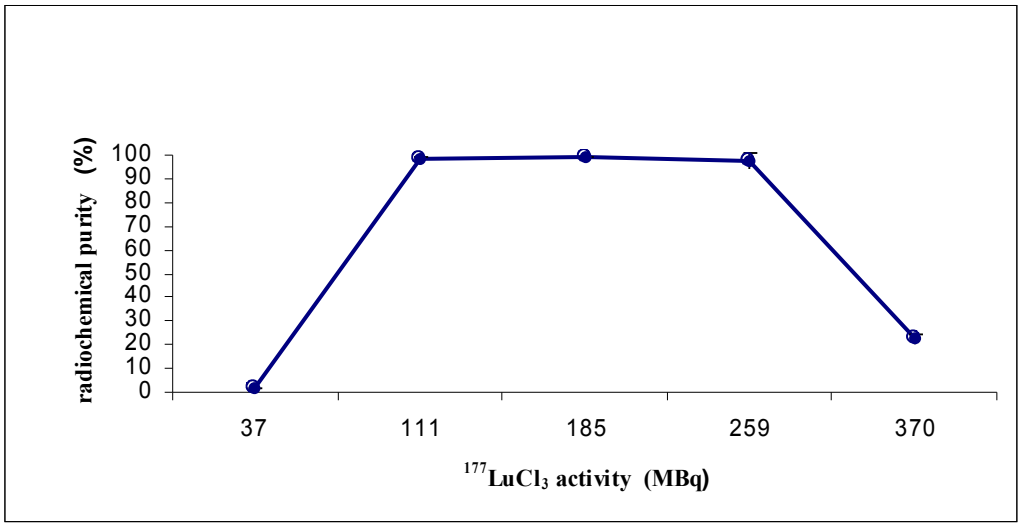

FIG. 3. The activity of ^{177}Lu on the radiochemical purity of the reactions

3.2. Stability of ^{177}Lu-DOTA-SP

The stability of labeled peptide was evaluated by instant thin layer chromatography after storage at 4 °C for different times and the results are shown in the Table III. The reactions proceeded at 90 °C for 30 minutes. For the point of 92.5MBq, the composition if kept approximately steady for 216 hours. In 185MBq the income was superior in the immediate control. While in the point of 377.5 MBq the composition lost the income in 24 hours.

Table III. *In vitro* stability of radiolabeled substance P after storing at 4 °C for different times.

Radiochemical Purity of ^{177}Lu-DOTA-Substance P				
10 µg of DOTA-SP, 92.5 MBq (2.5 mCi) of ^{177}LuCl$_3$				
Storage time at 4 °C				
Immediately	48 hours	72 hours	168 hours	216 hours
99.81 ± 0.1	98.36 ± 0.3	97.90 ± 0.3	95.58 ± 0.1	94.94 ± 0.4
10 µg of DOTA-SP, 185 MBq (5.0 mCi) of ^{177}LuCl$_3$				
Storage time at 4 °C				
Immediately	24 hours	72 hours	168 hours	216 hours

Radiochemical Purity of ^{177}Lu-DOTA-Substance P				
10 µg of DOTA-SP, 92.5 MBq (2.5 mCi) of ^{177}LuCl$_3$				
Storage time at 4 °C				
Immediately	48 hours	72 hours	168 hours	216 hours
99.81 ± 0.1	98.36 ± 0.3	97.90 ± 0.3	95.58 ± 0.1	94.94 ± 0.4
10 µg of DOTA-SP, 185 MBq (5.0 mCi) of ^{177}LuCl$_3$				
Storage time at 4 °C				
99.10 ± 0.5				
10 µg of DOTA-SP, 377.5 MBq (7.5 mCi) of ^{177}LuCl$_3$				
Storage time at 4 °C				
Immediately	24 hours	72 hours	168 hours	216 hours
96.08 ± 0.4	85.82 ± 5.7			

4. CONCLUSION

A high radiochemical purity (> 95%) was obtained and high specific activity of DOTA-SP was achieved when the reaction time was 30 minutes, the temperature was 90 ºC and the mass of DOTA-SP was 10 µg and ^{177}Lu activity was 185 MBq. These conditions extrapolate will be used in future experiments with high activity and also in *in vitro* and *in vivo* studies involving glioma models.

5. ACKNOWLEDGMENTS

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