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TECNETIUM-99m AS ALTERNATIVE TO PRODUCE SOMATOSTATIN-LABELED DERIVATIVES: COMPARATIVE BIODISTRIBUTION EVALUATION WITH ^{111}In-DTPA-OCTREOTIDE

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Abstract – Synthetic somatostatin (SST) analogues have been used in the preparation of receptor-specific radiopharmaceuticals for diagnostic and therapy of neuroendocrine tumors. This work studied the labeling conditions with ^{99m}Tc and biological distribution in *Swiss* mice of two SST analogs (HYNIC-Tyr^3-Octreotide and HYNIC-Tyr^3-Octreotate) and compared the biodistribution pattern with ^{111}In-DTPA-Octreotide. Biological distribution studies were performed after injection of radiopharmaceuticals on *Swiss* mice. Labeling procedures resulted on high radiochemical yield for all three preparations and the labeled products presented high *in vitro* stability. Biological distribution studies evidenced similar general biodistribution of ^{99m}Tc-labeled peptides when compared with indium-labeled peptide with fast blood clearance and elimination by urinary tract. Kidneys uptake of ^{99m}Tc-HYNIC-TATE are similar to ^{111}In-DTPA-Octreotide, and both are significantly higher than ^{99m}Tc-HYNIC-OCT. All labeled peptides presented similar uptake on liver, but the retention in time at intestines, particularly at large intestine, was more expressive for ^{111}In-labeled peptide. The %ID of ^{99m}Tc-HYNIC-OCT and ^{99m}Tc-HYNIC-TATE in organs with high density of SST receptors like pancreas and adrenals were significant and similar to obtained for ^{111}In-DTPA-Octreotide, confirming the affinity of these radiopharmaceuticals for the receptors.

Key words: ^{99m}Tc-HYNIC-Octreotide, ^{99m}Tc-HYNIC-Octreotate, ^{111}In-DTPA-OCT, neuroendocrine tumor, nuclear medicine.

INTRODUCTION

Nowadays, different diagnostic procedures can be applied to identify human diseases and pathologies, even before the appearance of the first symptoms. The success of the diagnostic procedures on Nuclear Medicine is in part due to the use of labeled biomolecules specifically targeted to an organ or a tumor. Scintigraphic images can be used to image the in vivo sites that express high density of specific receptors that recognize the labeled biomolecule (12). Labeled peptides have been extensively in oncology, neurology, cardiology and to identify infection/inflammation focus or trombous (1).

Somatostatin (SST) receptors have identified in different kinds of tumors such a neuroendocrine tumors and tumors of the central nervous system, breast, lung and lymphatic tissue making these receptors potential targets for radionuclide diagnostics and therapy (2).

Somatostatin is a cyclic hormone peptide with 14 amino acids and a short half-life in blood (approximately 3 minutes), being unstable *in vivo* and is therefore not suitable for application to diagnostic imaging. These observations have served as the biomolecular basis for the clinical use of radiolabeled SST analogues which, at present, are of great interest in nuclear medicine for diagnostic and peptide receptor radionuclide therapy (PRRT) applications.Octreotide (OCT), an octapeptide analog of SST, has a longer biological half-life, which makes it more suitable for labeling and imaging. OCT and other SST synthetic analogs like Tyr3-Octreotate (TATE), Lanreotide, Tyr3-Octreotide (OCT) and RC 160 have been used for radiopharmaceutical

production, labeled with different radionuclides (3,4).

The ^{111}In-DTPA-Octreotide (OctreoScan®) has found useful for imaging a range of tumours, including neuroendocrine cancer, carcinoide and lymphoma. However, ^{111}In has several drawbacks, such as a long half-life (67 h) and a suboptimal gamma energy (173 keV 89% and 247 keV 94%), which results in a low injectable dose and a relatively high radiation burden to the patient (5,7,8).

The radionuclide of choice would be ^{99m}Tc, produced by a radionuclide generator and therefore daily available, with 6 h half-life and 140keV monoenergetic gamma-ray emission ideal for conventional Nuclear Medicine imaging procedures (8).

As a consequence, the search for a technetium-99m-based somatostatin analogue has been intensified recently, but only one analog, 99mTc-depreotide (NeoTec) has so far been commercially introduced into clinical practice and this compound, however, does not have similar imaging properties as ^{111}In-DTPA-OCT. (5,6,7,8).

Recently, it was reported that Tyr^3-octreotate (TATE) showed improved binding to SST receptors sub type 2 when compared with Tyr^3-octreotide (OCT). The labelling of OCT and TATE with ^{99m}Tc has been described using the chelating group HYNIC (hydrazinonycotinic acid) and different coligands as EDDA (N,N' ethylenediaminediacetic acid) and tricine [9,12].

This work studied the labeling of HYNIC-TATE and HYNIC-OCT with ^{99m}Tc and biological behavior of the labeled compounds in animal model in order to compare the potential of these technetium-labeled peptides with the ^{111}In-DTPA-OCT derivative.

MATERIAL AND METHODS

Labelling Procedures

The labelling of DTPA-OCT (Pichem) with 111-indium ($^{111}InCl_3$, Nordion, Canada) were based in previously described procedures (1,2). The labelling of DTPA-OCT was performed at pH 4.5 in sodium acetate buffer, at room temperature for 30 minutes, using 10 ug of peptide and 37MBq of ^{111}In. (1). The stability of the preparations was evaluated over 48 hours.

The labelling of HYNIC-OCT and HYNIC-TATE (Anaspec, USA) with [Na^{99m}TcO4] (IPEN-TEC generator, Brazil) were performed using EDDA and tricine as coligands (7). Four labelling solutions were prepared: (A) Tricine: 60 mg of tricine/1.5 ml of 0.2N phosphate buffer pH 6.2; (B) EDDA: 30 mg of EDDA/1.5 mL of 0,1N NaOH; (C) Mixture: 1 mL of solution (A) + 1 mL of solution (B) and (D) $SnCl_2$: 10 mg of $SnCl_2.2H_2O$/10 mL of 0.1N HCl previously nitrogenated. For labelling procedure, in a reaction vial was introduced 20µg of HYNIC-OCT or HYNIC-TATE, followed by 1 mL of solution (C), 1 mL of pertechnetate solution (1110 MBq) and 15 µL of the solution (D). The reaction was conduted at 100°C for 10 minutes. The stability of the preparations was evaluated by 5 hours.

Quality Control

Radiochemical purity of indium-labelled peptide was determined by Instant Thin Layer Chromatography (ITLC-SG) using 0.1M sodium citrate buffer pH 5.5 as solvent. Radiochemical purity of technetium-labelled peptides was determined by ITLC-SG using different solvents to determine the radiochemical species as described in Table 1.

Table 1. ITLC-SG Chromatographic systems

	Espécie Radioquímica – Rf			
Solvente	**TcO_4**	**TcO_2**	**Tc-coligante**	**Tc-peptideo**
Metiletilcetona (MEK)	1	0	0	0
Citrato de Sódio 0.1M pH 5.0	1	0	1	0
Metanol : acetato de amonia (1 :1)	1	0	1	1

Biodistribution Studies

Biological distribution studies were developed in adults, normal *Swiss* mice (25-30g). The radiopharmaceuticals were injected in the tail vein (1.48 MBq/0.1 mL). The animals were sacrificed at 1.0 and 4.0 hours after the dose administration for indium-labelled peptide and at 1.0 and 5.0 hours for technetium-labelled peptides and the organs of interest were removed. The percent injected dose/organ (%ID) and percent injected dose/gram (%ID/g) were determined.

RESULTS

Tables 2 and 3 present the radiochemical purity of the ^{111}In and ^{99m}Tc preparations, and the stability in time. All the compounds were obtained in high radiochemical yield (superior

than 90%) and presented stability compatible with clinical application.

Table 2. Radiochemical purity (ITLC-SG) of DTPA-OCT labelled with 111-indium – stability of the preparations stored at room temperature

Time after labelling	Radiochemical purity: % of ^{111}In-DTPA-OCT
Immediately	99.55 ± 0.02
24 hours	96.62 ± 0.08
48 hours	97.34 ± 1.30

Table 3. Radiochemical purity (ITLC-SG) of HYNIC-OCT and HYNIC-TATE labelled with 99m-technetium – stability of the preparations stored at room temperature

Time after labelling	% [^{99m}Tc]HYNIC-TATE	% [^{99m}Tc]HYNIC-OCT
Immediately	96.00 ± 0.03	94.26 ± 0.56
5h	94.48 ± 0.33	93.48 ± 0.42

n = 3

Biodistribution studies of [^{111}In]DTPA-OCT in normal Swiss mice (Table 4) are compatible with the well known biodistribution pattern of this labeled compound, with fast blood cleareance, and relavely high uptake on kidney and significative uptake on intestine, especially in large intestine, 4 hours after the dose administration.

Comparatively, the HYNIC-somatostatin derivatives labeled with 99m-technetium showed similar biodistribution, with fast blood clearance (after 1 hour only about 1% of the administered dose is presented in blood), low uptake on stomach and thyroid and low uptake on liver and intestines. The two compounds also showed a low uptake in the body of the abdominal region relative low uptake in normal organs at abdominal region (stomach, liver and intestines).

DISCUSSION

Comparatively, the HYNIC-somatostatin derivatives labeled with 99m-technetium showed similar biodistribution, with fast blood clearance (after 1 hour only about 1% of the administered dose is presented in blood), low uptake on stomach and thyroid, that indicate high *in vivo* stability of both compounds (no free pertechnetate) and low uptake on liver and intestines. The relative low uptake in normal organs at abdominal region (stomach, liver and intestines) is specially favorable, contributing to the diagnostic of neuroendocrine tumors in this region.

High kidney uptakes were observed for ^{111}In-DTPA-OCT and ^{99m}Tc-HYNIC-TATE, probably due to the renal elimination of these low molecular weight peptides. The kidneys represent the critical organs for dosimetry when using labelled peptides, in this case, particularly to ^{111}In-DTPA-TOC, for which the renal uptake remained high after 4 hours of the dose administration (Table 7).

However, the renal uptake of the ^{99m}Tc-HYNIC-OCT compound was very low when compared with the two other compounds. It was recently reported (12) that the kidneys present high density of somatostatin receptors, specially of sst2 and sst5 types, that can contribute to explain the higher uptake of the HYNIC-Octreotate when compared to HYNIC-Octreotide. In this case, the high renal uptake of ^{111}In-DTPA-OCT can be related not only with the interation with sst receptors in the kidney but also with chemical properties of the labelled compound. The biological results in this study suggest that particular chemical configuration of the compounds when using different chelating groups and different radionuclides can promote differences in biodistribution patterns.

When considering the organs with high density of SST receptors, like adrenals and pancreas, the uptake of the ^{99m}Tc-labeled compounds were similar to 111In-DTPA-OCT (Tables 4, 5, 6).

The biological comparative distribution studies suggest that both ^{99m}Tc-labeled SST derivatives could be applied in diagnostic studies

Table 4. Biodistribution of ^{111}In-DTPA-OCT in normal Swiss mice (%ID/ organ and %ID/gram)

Organ	1 hour		4 hours	
	% ID/organ	*% ID/gram*	*% ID/organ*	*% ID/gram*
Brain	0.025 ± 0.005	0.06 ± 0.01	0.0097 ± 0.0011	0.03 ± 0.01
Thyroid	0.03 ± 0.01	-	0.018 ± 0.007	-
Lung	0.97 ± 0.05	3.78 ± 0.67	0.72 ± 0.05	2.95 ± 0.71
Heart	0.04 ± 0.01	0.36 ± 0.05	0.016 ± 0.003	0.12 ± 0.09
Spleen	0.05 ± 0.01	0.67 ± 0.19	0.06 ± 0.03	0.75 ± 0.29
Liver	0.55 ± 0.18	0.42 ± 0.11	0.45 ± 0.06	0.41 ± 0.05
Stomach	2.40 ± 0.43	8.48 ± 1.47	1.88 ± 0.37	7.78 ± 0.77
Muscle	0.06 ± 0.03	0.005 ± 0.002	0.025 ± 0.010	0.003 ± 0.001
Kidneys	19.12 ± 2.42	53.55 ± 4.84	21.14 ± 2.53	62.58 ± 5.00
S. intestine	2.45 ± 0.22	1.83 ± 0.25	4.06 ± 1.04	4.10 ± 1.28
L. intestine	1.13 ± 0.13	1.32 ± 0.17	4.85 ± 0.89	7.32 ± 1.01
Adrenals	0.053 ± 0.016	-	0.050 ± 0.010	-
Pancreas	1.65 ± 0.39	4.01 ± 0.80	0.98 ± 0.29	2.64 ± 0.54
Bone	0.08 ± 0.03	1.05 ± 0.37	0.05 ± 0.01	0.30 ± 0.22
Blood	1.02 ± 0.15	0.55 ± 0.07	0.23 ± 0.02	0.12 ± 0.02

(n=4)

Table 5. Comparative distribution in Swiss mice of ^{99m}Tc-HYNIC-TATE and ^{99m}Tc-HYNIC-OCT - % ID/organ

Organ	*^{99m}Tc-HYNIC-TATE* % ID/organ		*^{99m}Tc-HYNIC-OCT* % ID/organ	
	1 hour	**5 hours**	**1 hour**	**5 hours**
Lung	0.90 ± 0.22	0.56 ± 0.22	1.22 ± 0.11	0.70 ± 0.04
Heart	0.05 ± 0.01	0.01 ± 0.00	0.04 ± 0.01	0.013 ± 0.01
Spleen	0.04 ± 0.01	0.01 ± 0.00	0.04 ± 0.01	0.023 ± 0.01
Liver	0.40 ± 0.07	0.19 ± 0.07	0.51 ± 0.08	0.29 ± 0.01
Stomach	1.70 ± 0.31	0.59 ± 0.31	1.52 ± 0.14	1.11 ± 0.24
Kidneys	26.05 ± 4.59	16.28 ± 8.03	4.17 ± 0.70	1.62 ± 0.08
S. Intestine	2.80 ± 0.19	1.96 ± 0.50	3.04 ± 0.40	1.28 ± 0.20
L. Intestine	0.94 ± 0.12	1.76 ± 0.72	0.98 ± 0.02	3.08 ± 0.47
Total blood	0.74 ± 0.48	0.25 ± 0.03	1.18 ± 0.25	0.35 ± 0.03
Adrenals	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.017 ± 0.002
Pancreas	1.07 ± 0.33	0.47 ± 0.15	0.93 ± 0.22	0.45 ± 0.03
Thyroid	0.01 ± 0.00	0.01 ± 0.00	0.015 ± 0.003	0.015 ± 0.003

(n=4)

Table 6. Comparative distribution in Swiss mice of [^{99m}Tc]HYNIC-TATE and [^{99m}Tc]HYNIC-OCT - % ID/gram

Organ	*[^{99m}Tc]HYNIC-TATE % ID/gram*		*[^{99m}Tc]HYNIC-OCT % ID/gram*	
	1 hour	**5 hours**	**1 hour**	**5 hours**
Lung	3.13 ± 0.41	1.41 ± 0.47	5.50 ± 1.39	2.57 ± 0.83
Heart	0.23 ± 0.09	0.05 ± 0.03	0.27 ± 0.05	0.10 ± 0.02
Spleen	0.45 ± 0.12	0.12 ± 0.08	0.46 ± 0.08	0.32 ± 0.01
Liver	0.38 ± 0.30	0.11 ± 0.05	0.36 ± 0.05	0.18 ± 0.05
Stomach	5.14 ± 0.51	1.99 ± 1.35	4.44 ± 0.36	2.97 ± 0.74
Kidneys	49.70 ± 7.80	26.86 ± 12.65	10.05 ± 1.50	4.23 ± 0.07
Intestine (fine)	1.37 ± 0.11	0.98 ± 0.33	2.04 ± 0.09	1.00 ± 0.11
Intestine (large)	0.93 ± 0.19	1.62 ± 0.88	1.15 ± 0.09	3.30 ± 0.88
Blood/mL	0.39 ± 0.17	0.08 ± 0.01	0.49 ± 0.07	0.16 ± 0.01
Pancreas	2.86 ± 0.95	0.93 ± 0.33	3.06 ± 0.06	1.46 ± 0.91
Muscle	0.002 ± 0.001	0.001 ± 0.000	0.10 ± 0.01	0.05 ± 0.01

(n=4)

Table 7. Comparative renal uptake of ^{111}In-DTPA-OCT, ^{99m}Tc-HYNIC-OCT and ^{99m}Tc-HYNIC-TATE

Time (hour)	*RENAL UPTAKE (% ID/organ)*		
	^{99m}Tc-HYNIC-TATE	**^{99m}Tc-HYNIC-TOC**	**^{111}In-DTPA-TOC**
1	26.05 ± 4.59	4.17 ± 0.70	19.12 ± 2.42
4	-	-	21.14 ± 2.53
5	16.28 ± 8.03	1.62 ± 0.08	-

for localization and staging of neuroendocrine tumors.

The physical properties of technetium-99m favor its use when compared with the physical properties of the indian-111

Other articles in this theme issue include references (13-20).

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