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PRELIMINARY STUDIES OF EDDA-TRICINE-HYNIC-[Tyr^3]OCTREOTIDE LABELLED WITH TECHNETIUM-99m: RADIOPHARMACEUTICAL DEVELOPMENT FOR THE DIAGNOSTIC OF NEUROENDOCRINE TUMOURS

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ABSTRACT

The use of labelled molecules with high specificity for an organ or receptor in scintigraphy, generate good local images of these specific receptors that are expressed for the biomolecule in question, minimizing the exposition of other organs. Small labelled peptides have showed a big potential for tumors image and other diseases in nuclear medicine. The octreotide was the first somatostatin synthetic analog introduced in clinical use in the localization of tumours with superexpression of somatostatin receptors which is a hundred times over in tumors cells that in normal cells. It did many attempts to development of a somatostatin analog labelled with ^{99m}Tc utilizing a variety of chelant systems until development the HYNIC-D-Phe^1-Tyr^3-octreotide, using tricine and EDDA as coligands, showing maintenance of *in vivo* affinity and promising of biodistribution in animals with induced tumours.

This work involved the development of a ^{99m}Tc (technetium-99m) radiopharmaceutical based in a somatostatine peptide derivative (Tyr^3-octreotide, TOC), with HYNIC chelating group, to be applied in the diagnostic of neuroendocrine tumors in nuclear medicine. Quality control methodologies to be applied in determination of the radiochemical purity of the labelled compound was also studied as well the biodistribution in normal *Swiss* mouse.

The ^{99m}Tc-HYNIC-TOC was obtained in high radiochemical yield. Biodistribution studies suggests the potential of this radiopharmaceutical in the diagnostic of neuroendocrine tumours.

1. INTRODUCTION

Radiopharmaceuticals are drugs containing a radionuclide used in the clinic routine for diagnostic and therapy of many diseases in nuclear medicine. About 80 % of the radiopharmaceuticals used in nuclear medicine are ^{99m}Tc labelled compounds commercially available by ^{99}Mo-^{99m}Tc (molibdenium-99-technetium-99m) generators with low cost[1]. The ^{99m}Tc shows favorable characteristics for the utilization in diagnostic: 6 hours of short half-life, monoenergetic gamma rays emission of 140 keV ideal for scintigraphy images

proceedings in gamma chamber or in SPECT (Single Photon Emission Computed Tomography) in nuclear medicine[2] and particulate emission absent.

The use of labelled biomolecules, with high specificity for an organ or receptor, by scintigraphy, generate local good images of these specified receptors that are expressed for the biomolecule in question, minimizing the exposition of others organs[3]. Small labelled peptides have showed a big potential for tumors image and others diseases in nuclear medicine. The disponibility by synthetic source, high affinity for target tissue receptors and small size leaving to a rapid excretion, did peptides one of the primary targets in radiopharmaceuticals research[4]. The somatostatin is a peptide with fourteen aminoacids, multifunctional, synthesized by hipotalamo and pancreas, acting as neurotransmiter in central nervous system and as hormone in others locals[5]. Until this time, five types of somatostatin receptor (sstr) was identified. The superexpression of somatostatin receptors in tumours cells is a hundred times over that in normal cells[3], considering neuroendocrine tumours as carcinoides, pancreatic island cell tumour, small cell lung cancer and some thyroid carcinomas[5]. The somatostatin show affinity for all receptors while the synthetic analogs show a substantial variation[6]. The octreotide (TOC) was the first somatostatin synthetic analog introduced in clinical use[5].

The ^{111}In-DTPA-octreotide (octreotide-diethilene tetramine pentacetic acid-indium-111) has been applied in clinical, specially in oncology. However, some limitations as image properties and cost stimulated the search of new radionuclides as ^{90}Y (ytrium-90) and ^{161}Tb (terbium-161) for therapeutic application and, ^{68}Ga (galium-68), ^{64}Cu (copper-64), ^{18}F (fluor-18) and, in particular ^{99m}Tc, for image[7]. It did many attempts to development of a somatostatin analog labelled with ^{99m}Tc utilizing a variety of chelant systems until development of the HYNIC-D-Phe^1-Tyr^3-octreotide (6-hidrazino nicotinic acid-D-Phe^1-Tyr^3-octreoide) (figure 1), utilizing tricine and EDDA (ethilenediamine-N,N'-diacetic acid) as coligands, showing a maintenance of *in vivo* affinity in rats brain cortex membranes and promising patterns of biodistribution *in vivo* in animals with induced tumours, like tumour-kidney and tumour-liver relation bigger than ^{111}In-DTPA-octreotide[8].

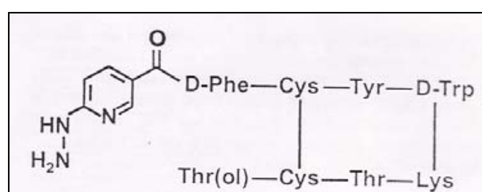

Figure 1. HYNIC-octreotide structure[8].

This work involves the development of a ^{99m}Tc radiopharmaceutical based in a somatostatin peptide derivative (Tyr^3-octreotide, TOC), with HYNIC chelating group, to be applied in the diagnostic of neuroendocrine tumours in nuclear medicine. Quality control methodologies to be applied in the determination of the radiochemical purity of the labelled compound will be also studied as well as the biodistribution of the labelled compound in normal *Swiss* mice.

2. MATERIALS AND METHODS

Labelling of HYNIC-TOC with ^{99m}Tc

In the reaction vial 20 µg of HYNIC-TOC in ethanol 10 % was introduced followed by, 10 mg of EDDA in NaOH 0.1 N, 20 mg of tricine in sodium phosphate buffer 0.2 N pH 6.2, 15 µg of $SnCl_2.2H_2O$ in HCl 0.1 N and 1110 MBq (30 mCi) of $^{99m}TcO_4^-$ and the reaction mixture was heated in boiling water bath for 10 minutes. This procedure was based in Guggenberg and col.[2, 10], Gabriel and col.[9] and, Decristoforo and col.[11] works with some modifications and was considered as the standard procedure. To optimize the reaction yield, many parameters was studied:
- reducing agent mass ($SnCl_2.2H_2O$): 30, 50, 60, 100, 150 and 167 µg
- coligands mass EDDA/tricine: 10/20 mg, 5/10 mg, 2,5/5 mg, 13,33/26,66 mg, 20/40 mg, and 10/0 mg
- peptide mass (HYNIC-TOC): 20 and 40 µg
- reaction time: 5, 10, 15 and 30 minutes
- temperature: room temperature and 100 ºC
- pH: 5.0, 6.0, 6.5, 7.5 and 8.0

Determination of radiochemical purity

It was possible to determine many radiochemical purity levels in the preparations, to contribute for the labelled optimization conditions of the peptide HYNIC-TOC with ^{99m}Tc (table 1).
The chromatographic system employed in the determination of the radiochemical purity of the preparations are described on table 1, considering the Rf for the different radiochemical species.

Table 1. Chromatographic system and relation front (Rf) of radiochemical species.

Radiochemical species	Stationary phase/Support	Movel phase/Solvent	Rf
$^{99m}TcO_4^-$	TLC-SG	Sodium citrate buffer	1
$^{99m}TcO_2$		0.1 N pH 5.0	0
^{99m}Tc-coligand			1
^{99m}Tc-HYNIC-TOC			0
$^{99m}TcO_4^-$	TLC-SG	Metilethilketone	1
$^{99m}TcO_2$			0
^{99m}Tc-coligand			0
^{99m}Tc-HYNIC-TOC			0
$^{99m}TcO_4^-$	ITLC-SG	Methanol:amonium acetate	1
$^{99m}TcO_2$		(1:1)	0
^{99m}Tc-coligand			1
^{99m}Tc-HYNIC-TOC			1

Biodistribution study

The radiopharmaceutical was administered in a dose of 1.48 MBq (40 µCi)/100 µL in a tail vein of the *Swiss* mice. After intervals of 1.5 h and 4 h, a heparinized capillar was used to collect a 100 µL aliquot of blood from the ocular plexus. The animals was sacrificed, the organs were removed (lung, heart, spleen, liver, stomach without content, thigh muscle, kidneys, small intestine with content, large intestine with content, adrenals and pancreas), weighed and the radioactivity in the organs and in the blood was measured at gamma counter. The tail was removed to corrected the administered dose.

The percentage of administered dose in different organs and in the blood was calculated as function of administered dose and expressed as % dose/organ and % dose/gram organ.

3. RESULTS AND DISCUSSION

Tables 2 to 6 are present the results of radiochemical purity in different reaction conditions, 30 minutes and 5 hours after labelling.

Table 2. Influence of $SnCl_2.2H_2O$ mass in labelling HYNIC-TOC with ^{99m}Tc at pH 6.5 using 20 µg HYNIC-TOC, 10 mg EDDA, 20 mg tricine, reaction time 10 min in water boiling bath.

		% radiochemical species		
$SnCl_2.2H_2O$ mass	Radiochemical species	30 min	5 h	№ assay
15 µg	$^{99m}TcO_4^-$	0.45 ± 0.26	1.95 ± 0.98	N= 6
(pattern condition)	$^{99m}TcO_4^-$ + ^{99m}Tc-coligand	5.66 ± 0.71	5.34 ± 1.23	
	^{99m}Tc-coligand	5.21 ± 0.59	3.39 ± 1.20	
	$^{99m}TcO_2$	2.23 ± 2.12	0.97 ± 0.62	
	^{99m}Tc-HYNIC-TOC	92.12 ± 2.53	93.69 ± 1.59	
30 µg	$^{99m}TcO_4^-$	7.47 ± 0.90	5.64 ± 0.59	N= 2
	$^{99m}TcO_4^-$ + ^{99m}Tc-coligand	12.50 ± 0.44	11.27 ± 0.43	
	^{99m}Tc-coligand	5.04 ± 0.46	5.63 ± 0.16	
	$^{99m}TcO_2$	1.50 ± 0.04	1.18 ± 0.14	
	^{99m}Tc-HYNIC-TOC	86.01 ± 0.40	87.56 ± 0.29	
60 µg	$^{99m}TcO_4^-$	5.30 ± 0.04	29.78 ± 1.32	N= 2
	$^{99m}TcO_4^-$ + ^{99m}Tc-coligand	14.21 ± 1.75	33.83 ± 1.85	
	^{99m}Tc-coligand	8.91 ± 1.79	4.05 ± 0.53	
	$^{99m}TcO_2$	2.43 ± 0.12	1.05 ± 0.16	
	^{99m}Tc-HYNIC-TOC	83.37 ± 1.87	65.13 ± 1.68	
100 µg	$^{99m}TcO_4^-$	3.88 ± 1.26	9.17 ± 0.91	N= 2
	$^{99m}TcO_4^-$ + ^{99m}Tc-coligand	19.49 ± 0.67	17.39 ± 0.26	
	^{99m}Tc-coligand	15.61 ± 0.59	8.22 ± 0.64	
	$^{99m}TcO_2$	2.61 ± 0.08	1.46 ± 0.09	

	Radiochemical species	30 min	5 h	№ assay
	^{99m}Tc-HYNIC-TOC	77.91 ± 0.76	81.16 ± 0.17	
150 µg	$^{99m}TcO_4^-$	3.11 ± 0.98	6.92 ± 1.46	N= 4
	$^{99m}TcO_4^-$ + ^{99m}Tc-coligand	15.95 ± 2.22	15.95 ± 2.49	
	^{99m}Tc-coligand	12.84 ± 3.02	9.03 ± 2.37	
	$^{99m}TcO_2$	2.43 ± 0.78	1.25 ± 0.24	
	^{99m}Tc-HYNIC-TOC	81.62 ± 1.74	82.80 ± 2.34	

Increasing the mass of $SnCl_2.2H_2O$ at pH 6.5, the percentage of labeled peptide decreases with increasing of formation of ^{99m}Tc-coligand and $^{99m}TcO_4^-$.

Table 3. Influence of $SnCl_2.2H_2O$ mass in labelling HYNIC-TOC with ^{99m}Tc at pH 8.0 using 20 µg HYNIC-TOC, 10 mg EDDA, 20 mg tricine, reaction time 10 min in water boiling bath.

		% radiochemical species		
$SnCl_2.2H_2O$ mass	Radiochemical species	30 min	5 h	№ assay
50 µg	$^{99m}TcO_4^-$	3.54 ± 0.35	4.06 ± 0.86	N= 2
	$^{99m}TcO_4^-$ + ^{99m}Tc-coligand	9.70 ± 0.60	7.54 ± 1.55	
	^{99m}Tc-coligand	6.16 ± 0.25	3.48 ± 2.40	
	$^{99m}TcO_2$	2.87 ± 0.02	1.95 ± 0.08	
	^{99m}Tc-HYNIC-TOC	87.44 ± 0.62	90.52 ± 1.63	
100 µg	$^{99m}TcO_4^-$	5.40 ± 0.56	8.31 ± 5.01	N= 2
	$^{99m}TcO_4^-$ + ^{99m}Tc-coligand	9.69 ± 0.68	13.77 ± 1.07	
	^{99m}Tc-coligand	4.30 ± 0.12	5.47 ± 6.09	
	$^{99m}TcO_2$	2.61 ± 0.43	2.67 ± 0.03	
	^{99m}Tc-HYNIC-TOC	87.71 ± 0.25	83.56 ± 1.05	
150 µg	$^{99m}TcO_4^-$	7.79 ± 4.18	8.19 ± 4.32	N= 4
	$^{99m}TcO_4^-$ + ^{99m}Tc-coligand	13.15 ± 2.89	14.67 ± 5.90	
	^{99m}Tc-coligand	5.36 ± 1.42	6.49 ± 2.07	
	$^{99m}TcO_2$	1.99 ± 0.06	1.78 ± 0.39	
	^{99m}Tc-HYNIC-TOC	84.86 ± 2.91	83.55 ± 5.74	
15 µg	$^{99m}TcO_4^-$	18.93 ± 0.42	14.80 ± 0.40	N= 2
40 µg of	$^{99m}TcO_4^-$ + ^{99m}Tc-coligand	20.22 ± 3.44	19.12 ± 0.69	
HYNIC-TOC	^{99m}Tc-coligand	2.74 ± 1.82	4.32 ± 0.29	
	$^{99m}TcO_2$	6.93 ± 0.06	3.65 ± 0.45	
	^{99m}Tc-HYNIC-TOC	71.41 ± 1.46	77.24 ± 0.24	

Increasing $SnCl_2.2H_2O$ mass at pH 8.0 , the percentage labeled peptide also decrease, but in less extension than at pH 6.5.

Table 4. Influence of mass of coligand in HYNIC-TOC labelling with technetium-99m at pH 6.5 using 20 µg HYNIC-TOC, 15 µg $SnCl_2.2H_2O$, reaction time 10 min in water boiling bath.

Coligand mass	% radiochemical species		
	30 min	5 h	№ assay
2.5 mg EDDA/5 mg tricine	88.40 ± 0.83	90.01 ± 1.32	N= 4
5 mg EDDA/10 mg tricine	90.53 ± 3.17	90.59 ± 1.87	N= 6
10 mg EDDA/20 mg tricine (pattern condition)	92.12 ± 2.53	93.69 ± 1.59	N= 6
10 mg EDDA/0 mg tricine	41.32 ± 2.81	51.36 ± 7.27	N= 2

The results obtained with the alteration of coligand mass evidenced the necessity of both coligands to promote good labelling conditions. The reduction of coligand mass decreased the radiochemical purity.

Table 5. Influence of temperature and reaction time in radiochemical purity of ^{99m}Tc-HYNIC-TOC using 20 µg HYNIC-TOC, 10 mg EDDA, 20 mg tricine, 15 µg $SnCl_2.2H_2O$.

Temperature/time	% radiochemical species		
	30 min	5 h	№ assay
Room temperature/10 min	76.96 ± 10.04	64.29 ± 28.35	N= 4
Water boiling bath/5 min	93.69 ± 1.65	91.80 ± 2.29	N= 4
Water boiling bath /10 min (pattern condition)	92.12 ± 2.53	93.69 ± 1.59	N= 6
Water boiling bath /15 min	88.07 ± 7.19	90.53 ± 2.43	N= 8
Water boiling bath /30 min	88.21 ± 0.47	89.32 ± 0.38	N= 2

Increasing the reaction time or using room temperature did not contribute to the labelling of peptide.

Table 6. Influence of pH on labelling HYNIC-TOC with technetium-99m, using 20 µg HYNIC-TOC, 10 mg EDDA, 20 mg tricine, 15 µg $SnCl_2.2H_2O$, reaction time 10 minutes in water boiling bath.

pH	% radiochemical species		
	30 min	5 h	№ assay
5.5	90.15 ± 0.86	91.20 ± 1.10	N= 2
6.0	91.67 ± 1.31	90.51 ± 0.56	N= 4
6.5	92.12 ± 2.53	93.69 ± 1.59	N= 6
7.5	94.72 ± 0.42	94.54 ± 0.41	N= 4

Radiochemical purity showed no significative increasing with the variation of pH.

Radiochemical purity determined in different experiments at 30 minutes and 5 hours showed, in general, the increasing in perant of $^{99m}TcO_4^-$ and decrease in percentage of ^{99}mTc-coligand. This fact can be explained by the relative instability of ^{99m}Tc-coligand specie, that results in $^{99m}TcO_4^-$ with the time. In some labelling, the percentage of ^{99m}Tc-HYNIC-TOC after 5 hours is growth than 30 minutes. Probably 30 minutes wasn't a sufficient time to obtain the maximum yield and the labelling may proceed in subsequent time.

Table 7. Biodistribution study by invasive method in *Swiss* mice, 1.5 hours and 4 hours after administration of the radiopharmaceutical in the tail vein, expressed as % dose/organ and dose/gram of organ.

	Time after administration			
	1.5 hour		4 hour	
Organ	% dose/organ	% dose/gram	% dose/organ	% dose/gram
Lung	1.22 ± 0.11	5.50 ± 1.39	0.70 ± 0.04	2.57 ± 0.83
Heart	0.04 ± 0.01	0.27 ± 0.05	0.013 ± 0.01	0.10 ± 0.02
Spleen	0.04 ± 0.01	0.46 ± 0.08	0.023 ± 0.01	0.32 ± 0.01
Liver	0.51 ± 0.08	0.36 ± 0.05	0.29 ± 0.01	0.18 ± 0.05
Stomach	1.52 ± 0.14	4.44 ± 0.36	1.11 ± 0.24	2.97 ± 0.74
Muscle	1.42 ± 0.16	0.10 ± 0.01	0.57 ± 0.03	0.05 ± 0.01
Kidneys	4.17 ± 0.70	10.05 ± 1.50	1.62 ± 0.08	4.23 ± 0.07
Small intestine	3.04 ± 0.40	2.04 ± 0.09	1.28 ± 0.20	1.00 ± 0.11
Large intestine	0.98 ± 0.02	1.15 ± 0.09	3.08 ± 0.47	3.30 ± 0.88
Adrenal	0.03 ± 0.01	-	0.017 ± 0.002	-
Pancreas	0.93 ± 0.22	3.06 ± 0.06	0.45 ± 0.03	1.46 ± 0.91
Thyroid	0.015 ± 0.003	-	0.015 ± 0.003	-
Blood	1.18 ± 0.25	0.49 ± 0.07	0.35 ± 0.03	0.16 ± 0.01

In the biodistribution by invasive method, the thyroid showed low uptake and demonstrate the *in vivo* stability of the compound. The blood clearance was rapid with rapid excretion by kidneys and relatively low uptake by liver, a positive aspect for the visualization of the abdominal region. The uptake on pancreas was high due to be the high concentration of sstr receptors.

4. CONCLUSIONS

The ^{99m}Tc-HYNIC-TOC was obtained in high radiochemical yield, using EDDA and tricine as coligand at pH 6.5. The easy access, adequate properties for images, with a short half-life,

low energy gamma and low cost became the ^{99m}Tc a radionuclide of the choice for the diagnostic of neuroendocrine tumours instead of ^{111}In.

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