COMPARISON OF ^{131}I-TYR^3-OCTREOTATE AND ^{131}I-DOTA-TYR^3-OCTREOTATE: THE EFFECT OF DOTA ON PHARMACOKINETICS AND STABILITY

E.B. DE ARAÚJO, E. MURAMOTO, L.T. NAGAMATI,
J.S. CALDEIRA FILHO, R.M. COUTO, C.P.G. SILVA
Institute of Energetic and Nuclear Research, IPEN-CNEN,
São Paulo, Brazil
Email: ebaraujo@ipen.br

Abstract

The authors compared the biodistribution, and in vivo and in vitro stabilities of ^{131}I-Tyr^3-octreotate and ^{131}I-DOTA-Tyr^3-octreotate. The peptides were radioiodinated by the chloramine T method and high radiochemical yields were obtained (greater than 97%). Both labelled compounds showed high stability when incubated in human plasma at 37°C. The ^{131}I-Tyr^3-octreotate showed significant hepatic uptake and biliary excretion. The biodistribution of ^{131}I-DOTA-Tyr^3-octreotate, however, can be compared with the distribution of radiometal labelled octreotide analogues.

1. INTRODUCTION

The introduction of radiolabelled somatostatin analogues for peptide recetor imaging and peptide receptor radiotherapy of neuroendocrine cancer has provided a primary focus of interest in nuclear medicine. The ^{111}In-DTPA-D-Phe^1-octreotide (OctreoScan) became the first radiopeptide to be routinely used for scintigraphy of somatostatin receptor positive tumours [1].

The introduction of the metal chelator DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) initiated a marked improvement in the stability of the radioconjugates, allowing the incorporation of a variety of radionuclides, such as ^{90}Y and ^{177}Lu, for receptor mediated therapy and ^{68}Ga and ^{64}Cu for positron emission tomography [2].

^{90}Y-DOTA-octreotide was tested in several clinical studies for use in treatment [3, 4]. One of the most recent developments is the introduction of ^{177}Lu-DOTA-Tyr^3-octreotate, in which the carboxy terminal threoninol has been replaced with the natural amino acid threonine, yielding a very high SSTR2 affinity [5].

For sst-target diagnosis and radiotherapy, radiohalogens offer a broad spectrum of suitable isotopes for single photon emission tomography (^{123}I),

positron emission tomography (^{18}F) and peptide receptor therapy (^{131}I, ^{125}I and ^{211}At). Consequently, ^{123}I labelled Tyr^3-octreotide was the first compound to be used for the imaging of somatostatine receptor positive tumours [6].

However, the experience gained with radioiodinated sst ligands showed that the diagnostic and therapeutic usefulness of these ligands was limited by their unfavourable biokinetics, in vivo deiodination and resulting dosimetry. Owing to fast hepatic uptake and biliary clearance, most of the tracers showed high abdominal background acitivity and fast blood clearance, leading to low tumour uptake. Additionally, they exhibited low tumour retention, which was often attributed to fast intracellular degradation of the tracers and subsequent extracellularization [7].

In this paper, the authors prepared radioiodinated octreotates, ^{131}I-Tyr^3-octreotate and ^{131}I-DOTA-Tyr^3-octreotate, with high radiochemical yield. Although the DOTA chelating group was not necessary to the radioiodination procedure, the authors evaluated the influence of the chelating group on biodistribution, particularly on hepatic uptake, biliary excretion and renal clearance. Tumour uptake was evaluated in nude mice bearing AR42J tumour.

2. MATERIALS AND METHODS

2.1. Reagents

DOTA-Tyr^3-octreotate was provided from piChem by the IAEA and the Tyr^3-octreotate was purchased from Anaspec (EUA). All other reagents were purchased from Sigma-Aldrich. [^{131}I]NaI was obtained from Nordion (Canada).

2.2. Radiolabelling

Radiolabelling of Tyr^3-octreotate and DOTA-Tyr^3-octreotate with ^{131}I- was performed using the chloramine T method. A solution of 10 μg of peptide in 40 μL of PBS (0.05M phosphate buffered saline, pH7.5) was transferred to the reaction vial. After addition of 10 μL (74–111 MBq) of radioiodine solution and 5 μL of chloramine T solution (1 mg/mL PBS), the cap was carefully stirred and the labelling reaction was allowed to proceed for 3 min at room temperature. To the reaction mixture, 5 μL of sodium metabisulphite solution (2 mg/mL PBS) was introduced as a reducing agent. Different molar peptide to radionuclide ratios were applied to the labelling of DOTA-Tyr^3-octreotate with ^{131}I.

2.3. Quality control

Radiochemical purity was determined by HPLC (Waters) using an RP C18 column (4.2 mm × 50 mm, 5 μm, Waters) with UV (230 nm) and radioactivity (Packard Canberra) detection, flow rate of 0.5 mL/min with a linear gradient of 40–80% (v/v) methanol in 50mM sodium acetate buffer (pH5.5) for 20 min, maintained for another 25 min. Free radioiodine was also determined by horizontal zone electrophoresis (Amersham Pharmacia) on Whatman 1 paper, 0.05M barbital buffer, pH8.6, 300 V, 40 min.

2.4. In vitro stability

The in vitro stabilities of ^{131}I-Tyr^3-octreotate and ^{131}I-DOTA-Tyr^3-octreotate were evaluated after incubation in human plasma at 37°C. Radiochemical purity was determined 1, 4 and 24 h after incubation using the electrophoresis procedure.

2.5. Animal studies

Biodistribution studies of ^{131}I-Tyr^3-octreotate and ^{131}I-DOTA-Tyr^3-octreotate were performed on normal Swiss mice and nude mice bearing AR42J rat pancreatic tumours. About 540 kBq/0.1 mL of the respective radiopharmaceutical were injected into the tail vein. The animals were sacrificed at different time points post-injection and the organs of interest were dissected. Tissue samples were weighed and the radioactivity was measured using a gamma counter (Packard). All experiments were carried out following the principles of laboratory animal care.

3. RESULTS AND DISCUSSION

3.1. Radioiodination

Labelled peptides were obtained with radiochemical purities exceeding 95%, as determined by the electrophoresis method.

The authors investigated different molar peptide to radionuclide ratios in order to obtain mono-iodinated peptides to be applied in the biodistribution studies, considering that the di-iodinated peptide no longer binds to the somatostatin receptor, as previously reported [8].

The HPLC profile of the ^{131}I-DOTA-Tyr^3-octreotate obtained when using a molar peptide to radionuclide ratio of 2.73 (7.4 MBq ^{131}I/μg peptide)

produced only one radiochemical species with a retention time of 22.73 min (Fig.1). When using a molar peptide to radionuclide ratio of 0.54 (37 MBq ^{131}I/μg peptide), a second radiochemical species can be observed in the HPLC profile (Fig. 2), with a retention time of 24.9 min, probably related to the di-iodinated form of the peptide.

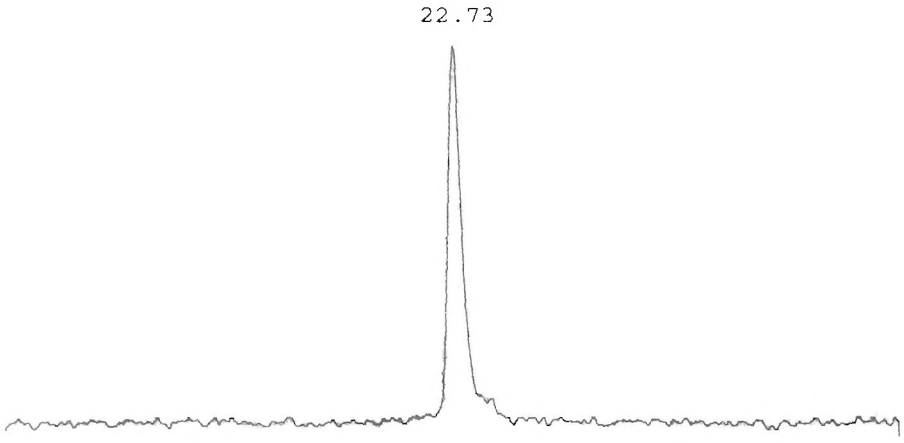

FIG. 1. HPLC profile of ^{131}I –DOTA-Tyr^3-octreotate molar peptide to radionuclide ratio of 2.73 (7.4 MBq/μg).

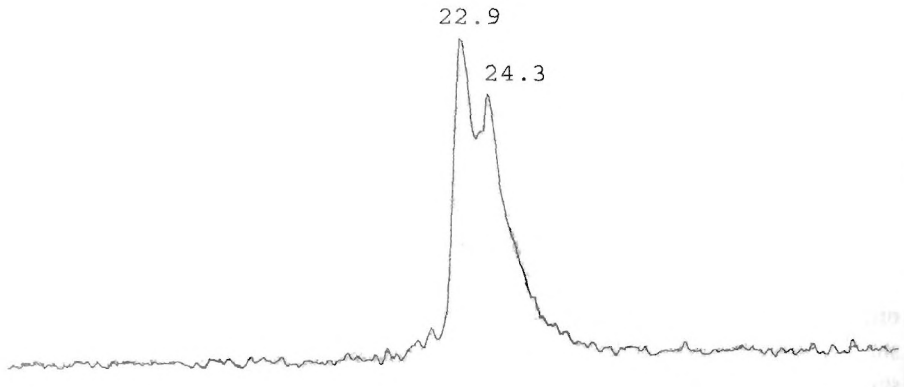

FIG. 2. HPLC profile of ^{131}I-DOTA-Tyr^3-octreotate molar peptide to radionuclide ratio of 0.54 (37 MBq/μg).

3.2. In vitro studies

In vitro studies show clearly the high level of stability of the iodinated ligands even after 24 h of incubation in human plasma (Table 1).

3.3. Biodistribution studies

Biological distribution studies were performed with radiolabelled peptides obtained in a peptide to radionuclide ratio of 2.73 with radiochemical purity exceeding 97%, which obviated the need to undertake the SepPak purification procedure. However, as with other radioiodinated peptides and proteins labelled on constituent tyrosine residues, it was important to study the possibility of dehalogenation in vivo.

The biodistribution data for ^{131}I-Tyr^3-octreotate (Table 2) was similar to that reported for ^{123}I-Tyr^3-octreotide in rats [9] and for ^{125}I-Tyr^3-octreotide and ^{125}I-Tyr^3-octreotate in nude mice [7]. The ^{131}I-Tyr^3-octreotate (Table 2) was extracted rapidly from the blood via hepatobiliary excretion, resulting in high liver uptake (2.28 ± 0.78%ID/g 1 h p.i.) and increasing intestinal uptake to 1 and 4 h p.i. (12.30 ± 4.36%ID/g for the small intestine and 14.85 ± 0.83%ID/g for the large intestine). The ^{131}I-DOTA-Tyr^3-octreotate was predominantly excreted via the kidneys. Nevertheless, renal activity accumulation for this compound was similar to that of ^{131}I-Tyr^3-octreotate.

TABLE 1. IN VITRO STABILITY OF ^{131}I-Tyr^3-OCTREOTATE AND ^{131}I-DOTA-Tyr^3-OCTREOTATE IN HUMAN PLASMA AT 37°C

Labelled peptide	Radiochemical purity (%)			
	Immediately	1 h	4 h	24 h
^{131}I-Tyr^3-octreotate	98.42 ± 0.32	98.43 ± 0.53	96.79 ± 1.01	95.76 ± 0.10
^{131}I-DOTA-Tyr^3-octreotate	95.41 ± 0.51	93.80 ± 0.80	92.40 ± 0.55	91.05 ± 0.55

TABLE 2. BIODISTRIBUTION OF ^{131}I-Tyr^3-OCTREOTATE AND ^{131}I-DOTA-Tyr^3-OCTREOTATE IN NORMAL SWISS MICE

Tissue	^{131}I-Tyr^3-octreotate Dose/g (%)			^{131}I-DOTA-Tyr^3-octreotate Dose/g (%)		
	1 h	4 h	24 h	1 h	4 h	24 h
Total blood	3.17 ± 0.32	0.81 ± 0.12	0.103 ± 0.006	2.56 ± 0.27	1.19 ± 0.13	0.020 ± 0.004
Liver	2.28 ± 0.78	0.42 ± 0.06	0.161 ± 0.034	0.70 ± 0.05	0.36 ± 0.05	0.088 ± 0.018
Spleen	0.76 ± 0.25	0.37 ± 0.11	0.114 ± 0.044	0.57 ± 0.06	0.27 ± 0.04	0.057 ± 0.018
Stomach	3.51 ± 1.67	1.94 ± 1.02	0.093 ± 0.032	3.32 ± 0.45	2.02 ± 0.59	0.19 ± 0.05
Int. (small)	12.30 ± 4.36	1.33 ± 0.96	0.045 ± 0.015	1.48 ± 0.13	0.57 ± 0.12	0.41 ± 0.19
Int. (large)	0.96 ± 0.58	14.85 ± 0.83	0.103 ± 0.032	0.44 ± 0.08	2.18 ± 0.25	1.90 ± 0.82
Kidney	12.54 ± 0.51	9.77 ± 3.46	3.752 ± 1.183	12.18 ± 0.86	9.86 ± 1.00	1.60 ± 0.22
Muscle	0.47 ± 0.24	0.13 ± 0.06	0.022 ± 0.002	0.26 ± 0.02	0.13 ± 0.03	0.03 ± 0.01
Brain	0.08 ± 0.01	0.024 ± 0.003	0.007 ± 0.001	0.08 ± 0.03	0.04 ± 0.010	0.006 ± 0.003
Heart	0.66 ± 0.11	0.20 ± 0.08	0.049 ± 0.020	0.50 ± 0.10	0.20 ± 0.05	0.019 ± 0.003
Lung	1.59 ± 0.40	0.45 ± 0.14	0.013 ± 0.021	0.80 ± 0.42	0.49 ± 0.22	0.052 ± 0.005
Thyroid *	0.49 ± 0.07	1.04 ± 0.20	0.878 ± 0.304	0.55 ± 0.10	1.23 ± 0.17	0.29 ± 0.06
Adrenals*	0.020 ± 0.007	0.007 ± 0.004	0.002 ± 0.0002	0.012 ± 0.003	0.008 ± 0.001	0.0014 ± 0.0005
Pancreas	0.87 ± 0.17	0.23 ± 0.05	0.023 ± 0.008	1.11 ± 0.52	0.79 ± 0.12	0.030 ± 0.010

* % dose organ; values are mean ± SD (n = 6).

Tracer uptake in the pancreas, tumour and adrenal were similar for both compounds ($p = 0.01$) after 1 h p.i. (Table 3). Tumour to blood ratios at 1 h p.i. were similar for both compounds but tumour to liver and tumour to intestine ratios were superior to ^{131}I-DOTA-Tyr^3-octreotate (Table 4).

Both labelled peptides presented low uptake in thyroid, which suggests low in vivo dehalogenation of the compounds.

The distribution pattern of ^{131}I-Tyr^3-octreotate was similar to that reported for ^{123}I-Tyr^3-octreotide in rats [9], with relatively high activity levels in liver and intestine. The biodistribution of ^{131}I-DOTA-Tyr^3-octreotate, however, can be compared with the distribution of radiometal labelled octreotide analogues in mice [10, 11].

TABLE 3. BIODISTRIBUTION OF ^{131}I-Tyr^3-OCTREOTATE AND ^{131}I-DOTA-Tyr^3-OCTREOTATE IN NUDE MICE BEARING AR42J RAT PANCREATIC TUMOURS

Tissue	^{131}I-Tyr^3-octreotate Dose/g (%)		^{131}I-DOTA-Tyr^3-octreotate Dose/g (%)	
	1 h	24 h	1 h	24 h
Total blood	2.12 ± 0.49	0.085 ± 0.035	2.93 ± 0.32	0.124 ± 0.006
Liver	2.07 ± 0.73	0.17 ± 0.03	1.34 ± 0.09	0.149 ± 0.009
Int. (small)	8.75 ± 1.87	0.061 ± 0.001	2.78 ± 0.60	0.067 ± 0.022
Muscle	0.37 ± 0.12	0.024 ± 0.009	0.42 ± 0.21	0.039 ± 0.013
Thyroid *	0.28 : 0.13	0.516 ± 0.031	0.54 ± 0.17	1.19 ± 0.31
Adrenals*	0.021 ± 0.010	0.002 ± 0.001	0.018 ± 0.004	0.003 ± 0.001
Pancreas	0.78 ± 0.05	0.038 ± 0.001	1.15 ± 0.29	0.047 ± 0.013
Tumour	1.10 ± 0.45	0.18 ± 0.08	1.73 ± 0.01	0.13 ± 0.01

* % dose organ; values are mean ± SD (n = 3).

TABLE 4. TUMOUR TO TISSUE RATIOS AT 1 h p.i. OF ^{131}I-Tyr^3-OCTREOTATE AND ^{131}I-DOTA-Tyr^3-OCTREOTATE IN NUDE MICE BEARING AR42J RAT PANCREATIC TUMOURS

Ratio	^{131}I-Tyr^3-octreotate	^{131}I-DOTA-Tyr^3-octreotate
Tumour to blood	0.51	0.59
Tumour to liver	0.53	1.29
Tumour to intestine (small)	0.13	0.62
Tumour to muscle	2.97	4.12

Although DOTA is not necessary for the radioiodination procedure, the chelating group seems to decrease the lipophilicity as evidenced by the low uptake in liver and intestines of ^{131}I-DOTA-Tyr^3-octreotate. In contrast to ^{131}I-Tyr^3-octreotate, which was eliminated via the hepatobiliary route, the DOTA analogue was predominantly cleared by the kidneys. The ^{131}I-DOTA-Tyr^3-octreotate also presented better tumour to non-tumour ratios, especially for liver and intestine, which are well known to be critical organs for scintigraphy (Table 4).

Radiometal labelled somatostatin derivatives often show longer tumour retention, compared to radioiodinated somatostatin analogues, due to intracellular trapping of the radionuclide or radiolabelled metabolites [7]. Despite this, the results obtained in this study with the ^{131}I-DOTA-Tyr^3-octreotate were promising and suggest the applicability of the compound for SPECT imaging or therapy using, respectively, ^{123}I or ^{131}I in labelling procedures.

ACKNOWLEDGEMENTS

This work was supported by IPEN-CNEN and IAEA.

REFERENCES

[1] KRENNING, E.P., et al., Somatostatin receptor scintigraphy with [^{111}In-DTPA-D-Phe^1]-octreotide in man: metabolism, dosimetry and comparison with [^{123}I-Tyr^3]-octreotide, J. Nucl. Med., **33** (1992) 652-658.

[2] CARSTEN, G., BERTRAN, W., Somatostatin receptor targeting for tumor imaging and therapy, Ann. N.Y. Acad. Sci., **1014** (2004) 258-264.

[3] WALDER, C., et al., Tumor response and clinical benefit in neuroendocrine tumors after 7.4 GBq ^{90}Y-DOTATOC, J. Nucl. Med., **43** (2002) 610-616.

[4] BODEI, L., et al., Receptor-mediated radionuclide therapy with ^{90}Y-DOTATOC in association with amino acid infusion: a phase I study, Eur. J. Nucl. Med., **30** (2003) 207-216.

[5] KWEKKEBOOM, D.J., et al., Treatment of patients with gastro-entero-pancreatic (GEP) tumours with the novel radiolabelled somatostatin analogue [^{177}Lu-DOTA0,Tyr^3]octreotate, Eur. J. Nucl. Med., **30** (2003) 417-422.

[6] KRENNING, E.P., et al., Localisation of endocrine-related tumours with radioiodinated analogue os somatostatin, Lancet, **1** (1989) 242-244.

[7] WESTER, H.J., et al., Comparison of radioiodinated TOC, TOCA and Mtr-TOCA: the effect of carbohidration on the pharmacokinetics, Eur. J. Nucl. Med., **29** (2002) 28-338.

[8] BAKKER, W.H., et al., Iodine-131 labelled octreotide:not an option for somatostatin receptor therapy, Eur. J. Nucl. Med., **23** (1996) 775-781.

[9] BAKKER, W.H., et al., Receptor scintigraphy with a radioiodinated somatostatin analogue: Radiolabeling, purification, biologic activity, and *in vivo* application in animals, J. Nucl. Med., **31** (1990) 1501-1509.

[10] AKIZAWA, H., et al., Renal metabolism of ^{111}In-DTPA-D-Phe^{1}-octreotide *in vivo*, Bioconjugate Chem., **9** (1998) 662-670.

[11] LEWIS, J.S., et al., *In vitro* and *in vivo* evaluation of ^{64}Cu-TETA-Tyr^{3}-octreotate. A new somatostatin analog with improved target tissue uptake, Nucl. Med. Biol., **26** (1999) 267-273.