MODIFIED BOMBESIN ANALOGUE WITH TECHNETIUM TRICARBONYL PRECURSOR AS PROSTATIC RADIODIAGNOSTIC AGENT

B.L. FAINTUCH*, E. MURAMOTO*, L. MORGANTI**,
R.M. CHURA-CHAMBI**, N. NASCIMENTO**,
E.G. GARAYOA***, P. BLAUENSTEIN***, P.A. SCHUBIGER***

*Radiopharmacy Center, Institute of Energetic and Nuclear Research
Email: faintuch@ipen.br

**Molecular Biology Center, Institute of Energetic and Nuclear Research

São Paulo, Brazil

***Center for Radiopharmaceutical Science, Paul Scherrer Institute,
Villigen, Switzerland

Abstract

Bombesin (BBN) and the molecularly related gastrin releasing peptide act as neurotransmitters and endocrine cancer cell growth factors on normal tissues as well as on neoplastic cells of various origins, including prostatic carcinomas and many breast carcinomas. The aim of the study was the evaluation of the labelling and biodistribution of modified BBN analogue with Tc-carbonyl core as a prostatic tumour diagnostic agent. BBN (7-14) was synthesized by substituting methionine (14) by norleucine and coupling the (NαHis)Ac ligand for application of the Tc-carbonyl labelling technique. Radiochemical evaluation was done and biodistribution studies were performed in normal Swiss mice at 1.5, 4 and 24 h post-injection, and in nude mice bearing prostate cancer cells PC-3, 1.5 h post-injection. The yield of the tricarbonyl intermediate was greater than 90%. Radiochemical purity for the radiolabelled BBN was 86.3 ± 1.2%. Biodistribution study results suggest that $^{99m}Tc(CO)_3$-BBN was mainly excreted by the hepato-biliary system and had high intestinal uptake. Tumour uptake was 1.15 ± 0.05% ID/g with tumour:blood and tumour:muscle ratios of 2.67 and 3.19, respectively. Scintigraphic imaging in nude mice bearing PC-3 cells showed a very low uptake by the tumour. Labelling conditions permitted a good yield. Nevertheless, the radiopharmaceutical did not show improved uptake by prostatic cell tumour, in comparison with findings observed without the modification in the molecule.

1. INTRODUCTION

Bombesin (BBN) is a 14 amino acid structure that belongs to the family of bombesin-like neuropeptides. It binds to receptors such as that of neuromedin B, the gastrin releasing peptide receptor and the orphan BBN subtype-3 receptor [1, 2]. BBN-like neuropeptides and their receptors also play a role in neoplasms. Stimulatory effects of the peptides on mitogenesis have been implicated in tumour growth of several human cancer cell lines such as lung, breast and prostatic cancers [3].

Blauenstein et al. have studied different radiolabelled BBN analogues [4–6]. Modifications of the BBN (7-14) molecule have been attempted in order to obtain derivatives that can increase plasma stability and provide easy labelling with radionuclides.

The finding that not only gastrin releasing peptide receptor is over expressed on human tumours but in some cases also neuromedin B and BB3 receptor subtypes led research groups to develop conjugates of the slightly modified BBN ligand [7].

The synthesis of Tc-carbonyl synthon has opened the door for ^{99m}Tc(I) peptide chemistry. The one step synthesis of the $[^{99m}Tc(CO)_3(H_2O)_3]^+$ complexes reported by Alberto and co-workers (1998) [8] by reduction of pertechnetate with sodium borohydride in aqueous solution in the presence of carbon monoxide, led to a new technique for development of biomolecules with radiopharmaceutical applications. Egli et al. (1999) have investigated the capability of amino acids and amino acid fragments to react with the ^{99m}Tc-tricarbonyl core [9]. It has been observed that ^{99m}Tc-tricarbonyl complexes which are coordinated with a tridentate chelating system exhibit good stability when challenged in human plasma, and also with excess cysteine, histidine or glutathione [10].

For radiolabelling the peptide with ^{99}Tc using the organometallic precursor $[^{99m}Tc(CO)_3(H_2O)_3]^+$, the peptide analogue was derivatized with a tridentate bifunctional chelator, Nα-histidinyl acetate.

2. OBJECTIVE

The aim of the study was the evaluation of the labelling and biodistribution of modified BBN analogue with ^{99m}Tc-carbonyl core as a prostatic tumour diagnostic agent.

The product was purified in a C18 SepPak cartridge before biological studies commenced. The impurities were eluted with water and $^{99m}Tc(CO)_3$-BBN with ethanol.

3.4. Biodistribution studies

Biodistribution studies were performed on normal Swiss mice (n = 3) at 1.5, 4 and 24 h post-injection and on nude mice bearing prostate cancer cells PC-3, 1.5 h post-injection. Scintigraphic images were documented for these last animals.

4. RESULTS AND DISCUSSION

The development of tracers based on BBN/GRP receptors for non-invasive scintigraphic evaluation would make possible the biochemical characterization of certain cancers and benefit patients by means of earlier therapeutic intervention. Previous studies [6, 9] of the $^{99m}Tc(CO)_3^+$ core demonstrated its usefulness as a convenient platform for drug development.

The precursor $^{99m}Tc(CO)_3(H_2O)_3^+$ and the radiolabelled peptide were evaluated using paper chromatography for the first and TLC-Al for the second in the same mixture solvent. The R_f of the radiochemical species can be seen in Table 1.

The yield of the tricarbonyl intermediate was greater than 90%. The radiochemical purity of the radiolabelled BBN was 86.3 ± 1.2%, with R_t = 19.1 (Fig. 1).

Biodistribution study results suggest that $^{99m}Tc(CO)_3$-BBN was mainly excreted by the hepato-biliar system and had high intestinal uptake as shown in Table 2. Tumour uptake was 1.15 ± 0.05% ID/g with tumour:blood and tumour:muscle ratios of 2.67 and 3.19, respectively (Table 3). Activity in the

TABLE 1. RETENTION FACTOR OF RADIOCHROMATOGRAM WHEN USING $^{99m}Tc(CO)_3^+$

Radiochemical species	Whatman no. 1	TLC-Al
$^{99m}TcO_4^-$	0.5–0.6	0.7
$^{99m}Tc(CO)_3$	0.8–0.9	0.0–0.2
$^{99m}TcO_2$	0.0	0.0
$^{99m}Tc(CO)_3$-conjugate	0.9–1.0	0.7

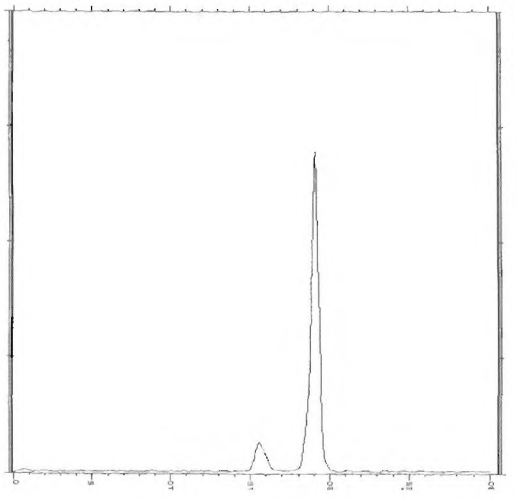

FIG. 1. Radiochromatogram of $^{99m}Tc(CO)_3$-BBN.

pancreas was used as a measure of receptor binding. At 1.5 h post-injection, the activity was only 1.23 ± 0.06% ID/g. There was no significant uptake or retention in the stomach indicating minimal, if any, in vivo dissociation for

TABLE 2. BIODISTRIBUTION (%ID/g) OF $^{99m}Tc(CO)_3$-BBN IN NORMAL SWISS MICE AS A FUNCTION OF TIME AFTER INTRAVENOUS ADMINISTRATION (n = 3)

Organ	1.5 h	4 h	24 h
Blood	0.48 ± 0.13	0.15 ± 0.02	0.06 ± 0.02
Heart	0.33 ± 0.50	0.26 ± 0.08	0.02 ± 0.01
Lung	0.81 ± 0.03	0.28 ± 0.03	0.10 ± 0.01
Kidney	1.97 ± 0.23	0.39 ± 0.01	0.12 ± 0.01
Spleen	0.53 ± 0.04	0.16 ± 0.05	0.04 ± 0.01
Stomach	0.97 ± 0.33	0.70 ± 0.16	0.18 ± 0.05
Pancreas	1.31 ± 0.04	0.43 ± 0.04	0.05 ± 0.02
Liver	5.26 ± 0.24	2.40 ± 0.23	0.57 ± 0.14
Large intestine	2.05 ± 1.78	3.01 ± 1.09	1.12 ± 0.06
Small intestine	4.60 ± 0.85	0.97 ± 0.17	0.11 ± 0.02
Muscle	0.50 ± 0.11	0.15 ± 0.01	0.01 ± 0.01

Note: Values represent mean ± SD (n = 3) of per cent of injected dose per gram.

TABLE 3. BIODISTRIBUTION OF ^{99m}Tc-$(CO)_3$-BBN IN PROSTATE TUMOUR BEARING NUDE MICE (PC-3) 1.5 h AFTER INTRAVENOUS ADMINISTRATION (n = 3)

Organ	1.5 h	
	%ID/g	%ID/organ
Blood	0.43 ± 0.05	0.63 ± 0.05
Heart	0.48 ± 0.06	0.04 ± 0.01
Lung	1.22 ± 0.14	0.22 ± 0.04
Kidney	2.34 ± 0.25	0.73 ± 0.12
Spleen	0.82 ± 0.32	0.12 ± 0.07
Stomach	0.56 ± 0.04	0.10 ± 0.01
Pancreas	1.23 ± 0.06	0.27 ± 0.05
Liver	5.82 ± 0.27	6.02 ± 0.12
Large intestine	2.36 ± 0.81	0.81 ± 0.11
Small intestine	5.72 ± 1.17	5.56 ± 0.28
Muscle	0.36 ± 0.14	0.05 ± 0.01
Tumour	1.15 ± 0.05	0.40 ± 0.10
Tumour:blood	2.67	
Tumour:muscle	3.19	

^{99m}Tc from these ligands to produce $^{99m}TcO_4^-$. Scintigraphic imaging in nude mice bearing PC-3 cells showed a very low uptake by the tumour (Fig. 2).

Efforts have been made to design derivatized BBN analogues for binding and pharmacokinetic studies. Because BBN agonists are generally preferable to BBN antagonists for receptor specific internalization, most BBN analogues with an amidated C-terminus are directly involved in the specific binding interaction with the gastrin releasing peptide receptor and the truncated C-terminal heptapeptide sequence (BBN(8-14)) must be maintained or minimally substituted. Blauenstein et al. (2004) [11] synthesized many BBN analogues and the substitution of Leu13 by cyclohexylalanin brought better stability of the complex in plasma. The authors have not tested stability in plasma but tumour uptake did not indicate that replacement of Met by norleucine was worthy. Along with insignificant pancreas uptake, that failure may be related to the capability of the derivative to target gastrin releasing peptide receptor expressing cells in vivo.

FIG. 2. Scintigraphic imaging of nude mice bearing prostate cancer tumour cells.

5. CONCLUSION

Labelling conditions permitted a good yield to be obtained. Substitution of the amino acid in position 14 by norleucine in the molecule had the advantage that no oxidation took place during synthesis and labelling, rendering it easier to work with this compound. Nevertheless, radiopharmaceutical analysis did not show improved uptake by prostatic cell tumour, in comparison with findings observed without this modification.

REFERENCES

[1] MAINA, T., et al., Species differences of bombesin analog interactions with GRP-R define the choice of animal models in the development of CRP-R-targeting drugs, J. Nucl. Med. **46** (2005) 823.

[2] FLEISCHMANN, A., WASER, B., GEBBERS, J.O., REUBI, J.C., Gastrin-releasing peptide receptors in normal and neoplastic human uterus: Involvement of multiple tissue compartments, The Journal of Clinical Endocrinology & Metabolism **90** 8 (2005) 4722.

[3] MOODY, T.W., CHAN, D., FAHRENKRUG, J., JENSEN, R.T., Neuropeptides as autocrine growth factors in cancer cells, Curr. Pharm. Des. **9** (2003) 495.

[4] BLAUENSTEIN, P., et al., Improving the tumor uptake of 99mTc-labeled neuropeptides using stabilized peptide analogues, Cancer Biotherapy & Radiopharmaceuticals **19** (2) (2004) 181.

[5] LA BELLA, R., et al., In vitro and in vivo evaluation of a 99mTc(I)-labeled bombesin analogue for imaging of gastrin releasing peptide receptor-positive tumors, Nucl. Med. Biol. **29** (2002) 553.

[6] LA BELLA, R., et al., A 99mTc(I)-postlabeled high affinity bombesin analogue as a potential tumor imaging agent, Bioconj. Chem. **13** 3 (2002) 599.

[7] ZHANG, H., et al., Synthesis and evaluation of bombesin derivatives on the basis of pan-bonbesin peptides labeled with Indium-111, Lutetium-177, and Yttrium-90 for targeting bombesin receptor-expressing tumors, Cancer Research **64** (2004) 6707.

[8] ALBERTO, R., SCHIBLI, R., EGLI, A., SCHUBIGER, A.P., "A novel organometallic aqua complex of technetium for the labeling of biomolecules: Synthesis of [99mTc(OH2)3(CO)3]+ from [99mTcO4]- in aqueous solution and its reaction with a bifunctional ligand", J. Am. Chem. Soc. **120** (1998) 7987.

[9] EGLI, A., et al., Organometallic Tc-99m-aquaion labels peptide to an unprecedented high specific activity, J. Nucl. Med. **40** (1999) 1913.

[10] SCHIBLI, R., SCHUBIGER, P.A., Current use and future potential of organometallic radiopharmaceuticals, Eur. J. Nucl. Med. **29** (2002) 1529.

[11] BLAUENSTEIN, P., et al., Improving the tumor uptake of 99mTc-labeled neuropeptides using stabilized peptide analogues, Cancer Biotherapy & Radiopharmaceuticals **19** 2 (2004).