2013 International Nuclear Atlantic Conference - INAC 2013
Recife, PE, Brazil, November 24-29, 2013
ASSOCIAÇÃO BRASILEIRA DE ENERGIA NUCLEAR - ABEN
ISBN: 978-85-99141-05-2

COMPARATIVE STUDY OF TWO DIFFERENT BOMBESIN DERIVATES LABELED WITH 111-In AND BIODISTRIBUTION IN NORMAL MICE

Ricardo S. Oliveira[1], Laís F. Alcarde[1], Beatriz L. Correa[1], Adriana V. F. Massicano[1], Renata M. Couto[1], Jair Mengatti [1], Elaine B. de Araújo[1]

[1] Instituto de Pesquisas Energéticas e Nucleares (IPEN / CNEN - SP)
Av. Prof. Lineu Prestes, 2242 – Cidade Universitária – CEP 05508-000
São Paulo – SP – Brasil
ricardooliveira@usp.br

ABSTRACT

Nuclear medicine is a medical speciality that uses radioactive compounds (radiopharmaceuticals), consisting of a substrate and a radioactive isotope, for diagnostic. Among the peptides of interest for Nuclear Medicine, bombesin (BBN), a 14 amino acid neuropeptide analog of human gastrin-releasing peptide, is one of the highlights. This is a comparative study aiming to establish the best condition to radiolabel two BBN derivatives, (DTPA-Phe-Gly_5-$BBN_{(6-14)}$) and (DTPA-Phe-Gly_2-$BBN_{(6-14)}$) with 111-indium. Specific objectives of this study were evaluate a good condition of radiolabelling in search excellent specific activity the bombesin derivatives and determinate the biodistribution in health mice model. Ten micrograms (10µg) of the derivative DTPA-Phe-Gly2-BBN (6-14) was labeled with 18.5 MBq (0.5 mCi) of $^{111}InCl_3$ at 25°C for different times (5, 15 and 30 minutes). The best condition was applied to peptide mass variation (10, 5, 2.5, 1, 0.5, 0.25 and 0.1 µg), keeping all other parameters fixed. Finally, the influence of $^{111}InCl_3$ activity in the radiolabeling process (18.5, 37, 55.5, 74, 185 MBq) was evaluated. The best conditions were repeated for the second derivate, DTPA-Phe-Gly_5-$BBN_{(6-14)}$. The radiochemical purity was assessed by thin layer chromatography (TLC), using 0.2 M EDTA pH 5 as solvent, and high performance liquid chromatography (HPLC) with a C18 column with linear gradient 10% A to 90% A (v/v) (A: 0,1% of TFA in CH3CN; B: 0,1% of TFA in H2O) at a flow rate of 1 mL/minute for 15 minutes. Considering the reaction time, the higher radiochemical purity was obtained when 10µg of the peptide was labeled with 18.5 MBq (0.5 mCi) of ^{111}In for 15 minutes at 25°C (97.33 ± 0.50%, n=3). In the mass variation study, the best results of radiochemical purity were obtained when 10 µg of the peptide was employed (97.69 ± 0.4%, n = 4). Finally, the maximum specific activity of the radiolabelled peptides was 1.85 MBq/ µg. The maximum specific activity is an important factor to be considered for studies in animals and to determine the potential of the radiopharmaceutical for clinical application. In this study, a standard labeling condition was determined, using 10 µg of peptide, 18.5 MBq (0.5 mCi) of $^{111}InCl_3$ for 15 minutes at 25°C for both peptides, and DTPA-Phe-Gly_5-$BBN_{(6-14)}$ showed good results in biodistribution.

1. INTRODUCTION

According to the National Institute of Cancer (INCA), for the biennium of 2012/2013 in Brazil, the most incident types of cancer were prostate and lung cancer in males and breast and cervical cancer in females. Staging of cancer is critical to the definition of prognosis and evaluation of response to therapy of the majority of patients, but the techniques available for

a clinic-pathological diagnosis are limited. With the evolution of researches in tumor markers, there have been grate advances in the field of molecular imaging of cancers, especially because cancer cell overexpress a number of specific antigens, also known as receptors that can differentiate them from normal cells. [1;2;3;4]

Peptides regulate the growth and function of cell intercommunication[5]. They can be used to identify the presence of specific receptors in the membrane of cancer cells and have the advantage of been easy to produce and having good pharmacokinetics characteristics. Bombesin is a 14 amino acid neuropeptide, analogous of the human gastrin-releasing peptide (GRP) and neuromedin B, isolated from the skin of the amphibian *Bombina bombina* and was first described in 1971[5]. The five cancers with the greatest epidemiological importance are known to overexpress the receptor for the GRP peptide and Bombesin derivatives (BBN) can be used for diagnostic with molecular imaging techniques using different radioisotopes or therapy.[6;7;8]

Nuclear Medicine or Molecular Image is a medical specialty that uses radioactive compounds (radiopharmaceuticals), consisting of a non radioactive molecule and a radioisotope, for diagnosis. Several radiopharmaceuticals consisting of radiolabeled proteins such as monoclonal antibodies and peptides are currently been studied.[9;10]

Several Bombesin derivatives with spacers of a different nature have already been radiolabeled with 111-indium, and the results were promising. 111-Indium (^{111}In) is a cyclotron radioisotope, produced by irradiation of 112-cadmium (^{112}Cd) with protons. It decays primarily by electronic capture with a physical half-life of 67.9 hours and its main feature photons emit 171 and 245 keV of energy.[11;12]

The specific objectives of this study were the evaluation of a good condition for radiolabeling with ^{111}In, in search excellent specific activity, two bombesin derivatives (DTPA-Phe-Gly_2-$BBN_{(6-14)}$ and DTPA-Phe-Gly_5-$BBN_{(6-14)}$), and to determinate the biodistribution in health mice model.

2. MATHERIALS AND METHODS

This work was conducted at the Radiopharmacy Center, Nuclear and Energetic Research Institute/Brazilian Commission of Nuclear Energy (IPEN/CNEN), São Paulo Brazil. All animal studies were performed at the Radiopharmacy Center, IPEN/CNEN, and the local animal welfare committee approved the protocol.

2.1 Chemicals and Analytical Methods

The DTPA-bombesin conjugates were synthesized by piCHEM (Austria). 111-Indium chloride ($^{111}InCl_3$) in 0.05 M HCl was purchased from Nordion (Canada). All other reagents

were purchased from Merck (Germany), with the following exceptions: trifluoroacetic acid (TFA), gentisic acid and methionine were from Sigma-Aldrich (USA) and Chelex 100 ion exchange resin was from BioRad (USA). All reagents were of analytical grade and the solvents for HPLC were HPLC grade.

Thin layer chromatography in silica gel 60 (TLC-SG, Merck, Germany) was applied to determine free indium in radiolabeling mixtures, with ethylenediaminetetraacetic acid (EDTA, 0.2M, pH5.0) as solvent.

Reversed-phase high-performance liquid chromatography (RP-HPLC) analyzes of $^{111}InCl_3$ and radiolabeled and non-radiolabeled DTPA-bombesin conjugates were performed on a Shimadzu system (Japan) equipped with an analytical reversed-phase C-18 column (4.0x150mm, 5 μm – Waters, USA); SPD-10A UV-vis (Shimadzu, Japan) absorbance detector (λ=280nm); a CTO-10 Avp column heater (Shimadzu, Japan) and a radiometric in-line Shell Jr. 1000/2000 (Shell-usa, USA) NaI solid scintillation detector. Method were used applying trifluoracetic acid 0.1% in water (A) and trifluoracetic acid 0.1% in acetonitrile (B) as solvents.

Ten micrograms (10μg) of the derivative DTPA-Phe-Gly_2-$BBN_{(6-14)}$ was labeled with 18.5 MBq (0.5 mCi) of $^{111}InCl_3$ at 25°C for different times (5, 15 and 30 minutes). The best condition was applied to peptide mass variation (10, 5, 2.5, 1, 0.5, 0.25 and 0.1 μg), keeping all other parameters fixed. Finally, with the best conditions, the influence of $^{111}InCl_3$ activity were evaluated in the radiolabeling process (18.5, 37, 55.5, 74, 185 MBq). The best labeling conditions were repeated for the second derivative, DTPA-Phe-Gly_5-$BBN_{(6-14)}$. The radiochemical purity was assessed by thin layer chromatography (TLC) and HPLC as previously described.

2.2 Animals

In vivo studies were performed in mice BALB-C, male, six to ten weeks of age and 20 to 25 grams of weight (Vivarium – IPEN).

3. RESULTS AND DISCUSSION

In the time variation study, the highest radiochemical purity was obtained when 10μg of the peptide was radiolabelled with 18.5 MBq (0.5mCi) for 15 minutes at 25°C (97.33%±0.50; n=3) (Table 1). With the time of the reaction determined, the mass of the peptide was evaluated as represented on Figure 1.

After the determination of the reaction time and the peptide mass, the ratio mass of peptide/$^{111}InCl_3$ activity was evaluated and results are presented in Table 2.

Table 1: Radiochemical purity (TLC-SG) after time variation (10µg DTPA-Phe-Gly_2-$BBN_{(6-14)}$ in acetate sodium buffer solution pH 4.5; 18.5MBq (0.5mCi) of $^{111}InCl_3$; 25°C; 350 rpm) (n=3)

Reaction time (minutes)	Radiochemical Purity (%)
5	95.10 ± 5.72
15	97.33 ± 0.50
30	97.43 ± 1.33

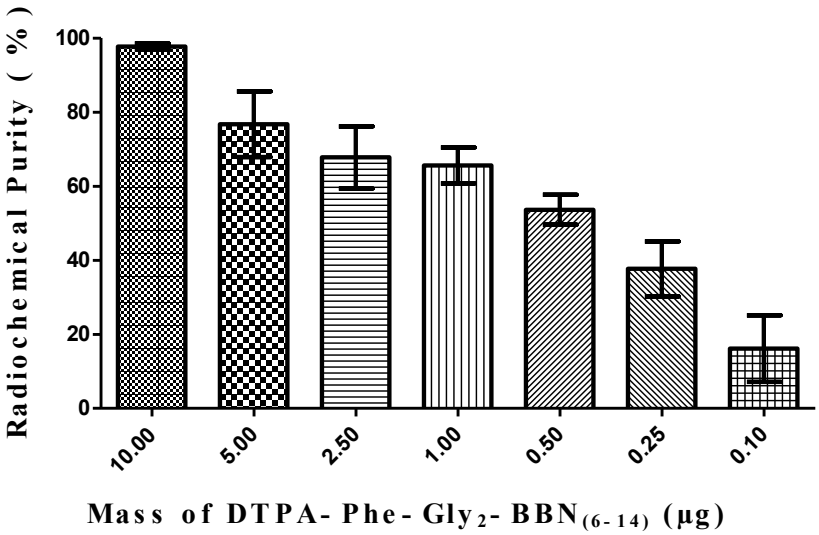

Figure 1: Effect of mass on the radiochemical purity (TLC) of the labeling reaction of DTPA-Phe-Gly_5-$BBN_{(6-14)}$ with 18.5 MBq (0.5 mCi) of $^{111}InCl_3$ (n = 4)

Table 2: Effect of the increasing activity on the radiochemical purity (TLC) of the radiolabeling reaction of DTPA-Phe-Gly_2-$BBN_{(6-14)}$ with $^{111}InCl_3$.

DTPA-Phe-Gly_2–$BBN_{(6-14)}$ (µg) mass	$^{111}InCl_3$ MBq/mCi activity	^{111}In-DTPA-Phe-Gly_2-$BBN_{(6-14)}$ (%) radiochemical purity	^{111}In-DTPA-Phe-Gly_2-$BBN_{(6-14)}$ (%) Radiochemical Purity
10	37 / 1	98.9±9.98 (n=3)	Nd
10	55.5 / 1.5	59.97±2.15 (n=3)	Nd
10	74 / 2	43.16±7.09 (n=3)	Nd
10	185 / 5	37.16±4.56 (n=3)	Nd
20	37 / 1	98.2 (0.2) (n=2)	99.00 (n=1)
20	92.5 / 2.5	98.47 (n=1)	nd
20	148 / 4	98.98 (n=1)	92.25 (n=1)

Nd = Not determined

After these experiments, the reaction time was fixed in 15 minutes, at 25ºC. According with the mass/activity ratio study, the best conditions were observed when radiolabeling 10µg of DTPA-Phe-Gly_2-$BBN_{(6-14)}$ peptide with 18.5 MBq (0.5mCi) and 20µg with 37 MBq (1 mCi) (specific activity 1.85 MBq/µg) (Figure 2).

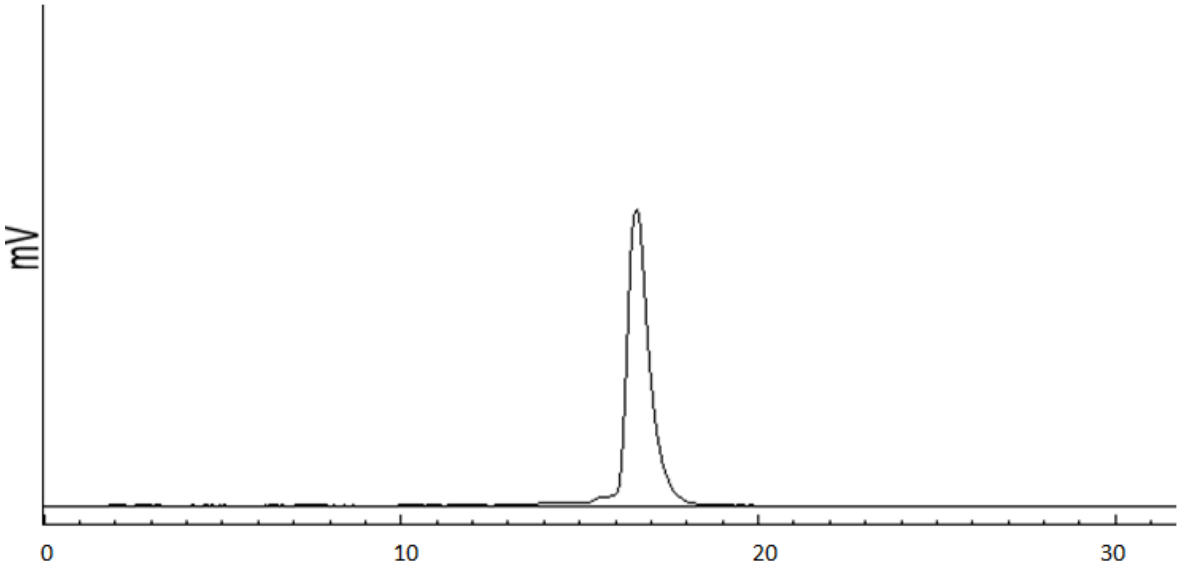

Figure 2: High Performance Liquid Chromatography (HPLC) (radioactive) of) ^{111}In-DTPA-Phe-Gly_2-$BBN_{(6-14)}$, with the specific activity of 1.85 MBq/µg, using C18 column, with linear gradient 10 a 90% (v/v) of 0.1% TFA in CH_3CN and 0.1% TFA in H_2O, flow rate of 1 mL/min for 20 minutes.

The activity of $^{111}InCl_3$ incorporated per mol of peptide, in other words, specific activity of the molecular marker, is an important characteristic for the development of new radiopharmaceuticals. From the clinical point of view, radiopharmaceuticals with a low specific activity are not so good, once the non-radiolabeled molecules compete with the labeled ones for the receptors *in vivo* and they cause more toxicity due to the biological effects of this binding. In other hand, specific activities too high are also prejudicial, once it causes radiolysis, and in the case of proteins, denaturation. The specific activity of Bombesin derivatives radiolabeled with ^{111}In described in the literature are variable.

After the determination of reaction time, activity of $^{111}InCl_3$ and peptide mass for the DTPA-Phe-Gly_2-$BBN_{(6-14)}$ (BBN2), the conditions were applied on the labeling of DTPA-Phe-Gly_5-$BBN_{(6-14)}$ (BBN5), and the biodistribution studies with both radiolabeled peptides were conducted on healthy animals. The results of both studies are shown on tables 3 and 4.

The derivative BBN2 presented bigger uptake in the bone than BBN5, suggesting that the use of a bigger spacer between the chelating group and the amino acid specific sequence provides a more stable radiolabeling peptide, since the free radionuclide accumulates in bones. With the biodistribution studies we could conclude that both peptides have low lipophilicity, since they do not cross the blood-brain-barrier and presented fast blood clearance. The results suggest that both peptides are excreted primarily by the kidneys, with low uptake on liver and intestines. It is known that the pancreas normally express BBN receptors, which works as a binding control of Bombesin derivatives, and it is responsible for the abdominal accumulation of this peptides. In our study, BBN5 showed a major uptake by the pancreas, indicating that a bigger spacer provides a better interaction between the radiolabeled peptide and the receptor.[11; 13; 14;15;16;17;18]

Table 3: Biodistribution in normal mice of ^{111}In-DTPA-Phe-Gly_2-$BBN_{(6-14)}$ (BBN2) at 1, 4 and 24h post-injection (n=3)

Organs	% ID per gram		
	Time 1h	Time 4h	Time 24h
Blood	0.58 ± 0.09	0.06 ± 0.01	0.00 ± 0.00
Heart	0.15 ± 0.07	0.03 ± 0.01	0.00 ± 0.00
Lungs	0.15 ± 0.12	0.11 ± 0.02	0.02 ± 0.01
Pancreas	0.17 ± 0.15	0.26 ± 0.37	0.00 ± 0.00
Spleen	0.10 ± 0.05	0.07 ± 0.02	0.01 ± 0.00
Stomach	0.20 ± 0.18	0.84 ± 1.41	0.02 ± 0.00
Liver	0.34 ± 0.24	0.35 ± 0.51	0.12 ± 0.14
Kidneys	1.78 ± 0.64	1.00 ± 0.64	0.32 ± 0.08
Intestine	0.24 ± 0.08	0.58 ± 0.20	0.04 ± 0.01
Muscle	0.53 ± 0.64	0.66 ± 1.12	0.00 ± 0.00
Bone	0.26 ± 0.16	0.04 ± 0.00	0.01 ± 0.01
Brain	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.0

Table 4: Biodistribution in normal mice of ^{111}In-DTPA-Phe-Gly$_5$-BBN$_{(6-14)}$ (BBN5) at 1,4, and 24h post-injection (n=3)

Organs	% ID per gram		
	Time 1h	Time 4h	Time 24h
Blood	0.09 ± 0.04	0.09 ± 0.07	0.03 ± 0.02
Heart	0.09 ± 0.04	0.02 ± 0.00	0.01 ± 0.00
Lungs	0.31 ± 0.08	0.06 ± 0.00	0.02 ± 0.00
Pancreas	0.53 ± 0.08	0.46 ± 0.21	0.16 ± 0.06
Spleen	0.10 ± 0.01	0.24 ± 0.34	0.03 ± 0.00
Stomach	0.15 ± 0.01	0.39 ± 0.55	0.02 ± 0.00
Liver	0.18 ± 0.05	0.06 ± 0.02	0.03 ± 0.00
Kidneys	2.06 ± 0.35	1.61 ± 0.37	0.35 ± 0.12
Intestine	0.11 ± 0.13	0.28 ± 0.16	0.03 ± 0.00
Muscle	0.08 ± 0.02	0.31 ± 0.22	0.00 ± 0.00
Bone	0.08 ± 0.04	0.16 ± 0.12	0.02 ± 0.16
Brain	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00

4. CONCLUSIONS

Bombesin derivatives could be labeled with 111-indiun in high specific activity (1.85 MBq/μg), as desired for peptide-receptor radiation diagnosis, and with high radiochemical yield. The peptide that showed better results in biodistribution studies with healthy mice was the BBN5 derivative, and its potential for tumor identification will be investigated in tumor model animals.

ACKNOWLEDGMENTS

The authors are grateful to CAPES/PROEX and Radiopharmacy Center/IPEN for financial support.

REFERENCES

1. “Incidência de Câncer no Brasil,” www.inca.gov.br/estimativa/2012/index.asp?ID=2 (2013).

2. R. A. Weinberg, *Biologia do câncer*. Artmed, Porto Alegre, BR, 2008.

3. A. Varvarigou, P. BOUZIOTIS, C. ZIKOS, F. SCOPINARO, G. DE VINCENTIS. “Gastrin-releasing peptide (GRP) analogues for cancer imaging.” *Cancer Biother Radiopharm,*Vol. 19,n. 2, p.219-229, 2004.

4. A. MEJIA, S. SCHULZ, T. HYSLOP, D.S. WEINBERG, S. A. WALDMAN. " Molecular sataging individualizing cancer management. *Journal of Surgical Oncology,* v.105, n. 5, p. 468-474.

5. A. ANASTASI, V. ERSPAMER, M. BUCCI. "Isolation and structure of Bombesin and alytesin, 2 analogous active peptides from the skin of the European amphibians Bombina and Alytes." *Experientia,* v. 27, n. 2, p. 166-167, 1971.

6. L.C. KNIGHT, Radiolabeled peptides for tumor imaging. In: M. J. WELCH and C.S. REDVANLY (Ed). *Handbook of radiopharmaceuticals: radiochemistry and applications.* Chichester: Wiley, 2013. Cap.23

7 . V. AMBROSINI, M. FANI, S. FANTI, F. FORRER, H.R. MAECKE. "Radiopeptide imaging and therapy in Europe" *Journal Nuclear Medicine*, v.52, p. 42S-55S, 2011.

8. R. E. WEINER, M. L. THAKUR. "Radiolabeled peptides in the diagnosis and therapy of oncological diseases." *Appl Radiat Isot,* v.57, n.5, p.749-763, 2002.

9. K. D. WEATHERMAN, W. CRISP, H. WEBER. The Physiological basis of radiopharmaceuticals. In: B. T. SMITH, *Nuclear Pharmacy.* London: Pharmaceutical Press, 2010. Cap.3, p.55-66.

10. B.T. SMITH. Radioactive drugs in medicine: radiopharmaceuticals. In: B. T. SMITH (Ed.) *Nuclear Pharmacy.* London: Pharmaceutical press, 2010. cap.1, p.1-12.

11. W. GALBRAITH, W. Radiopharmaceuticals in nuclear medicine. In B. T. SMITH (Ed). *Nuclear Pharmacy.* London: Pharmaceutical press, 2010, cap.6, p.111-186. ISBN 978 0 85369 866 1.

12. P.B. PUJATTI, "Marcadores moleculares derivados da bombesina para diagnóstico de tumores por SPECT e PET" 2012. 230 (Doutorado) Instituto de Pesquisas Energéticas e Nucleares, Universidade de São Paulo, São Paulo. www.pelicano.ipen.br/PosG30/TextoCompleto/Pricilla%20Brunelli%20Pujatti_D.pdf (2013)

13. G. B. SAHA, *Fundamentals of nuclear pharmacy,* 5 ed., Springer, New York, USA, 2003.

14. T. J. HOFFMAN, H. GALI, C. J. SMITH, G. L. SIECKMAN, D. L. HAYES, N. K. OWEN; W. A. VOLKERT. "Novel series of 111-In labeled bombesin analogs as potential radiopharmaceuticals for specific targeting of gastrin releasing peptide receptors expressedon human prostate cancer cells." *Journal Nuclear Medicine,* v.44, n.5, p.823-831, 2003

15. R. P. SCHRODER, C. MULLER, S. RENEMAN, M. L.MELIS, W.A. BREEMAN, E. BLOIS, C. H. BANGMA, E. P. KRENNING, W. M. VAN WEERDEN, M. DE JOG. "A standardised study to compare prostate cancer targeting efficacy of five radiolabelled Bombesin analogues." *European Journal Nuclear Molecular Imaging,* v.37, n.7, p.1386-1396, 2010.

16 . W. A. BREEMAN, M. DE JONG, J. L. ERION, J. E. BUGAJ, A. SRINIVASAN, B. F. BERNARD, D. J. KWEKKEBOOM, T. J. VISSER, E. P. KRENNING. “Preclinical comparison of (111)In-labeled DTPA- or DOTA- Bombesin analogs for receptor-targeted scintigraphy and radionuclide therapy.” *Journal Nuclear Medicine,* v. 43, n.12, p.1650-1656, 2002.

17. B. L. FAINTUCH, R. TEODORO, A. DUATTI, E. MURAMOTO, S. FAINTUCH, C. J. SMITH. “Radiolabeled Bombesin analogs for prostate cancer diagnosis: preclinical studies.” *Nuclear Medicine Biology,* v. 35, n. 4, p. 401-411, 2008.