2024 International Nuclear Atlantic Conference – INAC 2024
Escola de Guerra Naval, Rio de Janeiro, RJ, May 6 - 10, 2024

ISBN: 978-65-594-1256-3

Optimization of PSMA-I&T radiolabeling with 177Lu

Luiza M. Balieiro[1], Maria das D. Domingos[1], Luana P. da Silva[1] and Elaine B. de Araujo[1]

[1] Centro de Radiofarmácia
Instituto de Pesquisas Energéticas e Nucleares IPEN-CNEN
Av. Prof. Lineu Prestes, 2242 – Cidade Universitária – CEP 05508-000
São Paulo – SP – Brasil
luiza.mbalieiro@usp.br; mariadomingos0402@gmail.com; lua.pereira0656@gmail.com; ebaraujo@ipen.br.

1. Introduction

Prostate cancer (PCa) is the second most common type of cancer in men and the fifth cause of mortality worldwide. The International Agency for Research on Cancer estimated that by 2025, there is an incidence of 1.65 million men affected worldwide [1]. Metastatic prostate cancer is associated with a poor prognosis and decreased life expectancy. Usually, the treatment of choice is generally androgen deprivation therapy (ADT) and taxane-based chemotherapy, however, the problem related to this therapeutic approach is the potentially serious adverse effects, in addition, it is possible that patients develop castration-resistant prostate cancer (mCRPC), through mechanisms that are not yet very clear, representing a challenge in the search for new therapies that are safe, with low toxicity and favorable in terms of increasing overall survival [2,3,4,5].

Prostate-specific membrane antigen (PSMA) is a type II transmembrane glycoprotein that is anchored in the epithelial prostate cell membrane, overexpressed in prostate cancer [6], increased in metastatic castration-resistant patients and with a consensus that its expression level is linked to the malignancy of the disease [7,8,9].
Therefore, some radioligands for diagnosis and therapy of prostate cancer are being described based on the discovery of PSMA inhibitors, which specifically bind the Glu-urea-Lys pharmacophoric group, as is the case of PSMA-I&T for radiolabeling with lutetium-177 [9].

Lutetium-177 is a radioisotope produced in reactors, which application for therapy has been intensifying, due to its appropriated characteristics like $T_{1/2}$ of 6.65 days, E_{β} (max) 497 KeV (78,5 %) and 230 µm of medium range, making it ideal for the treatment of micro metastases, such as those that appear in castration-resistant prostate cancer [10].

This present work aims to determine the most favorable conditions for labeling PSMA-I&T with carried-added lutetium-177, to obtain a radiopharmaceutical with high radiochemical purity, avoiding the final purification step.

2. Methodology

2.1 Radiolabeling

The radiolabeling of PSMA-I&T (CMR-Russia) with lutetium trichloride ($^{177}LuCl_3$) (JSC, Russia or Isotopia, Israel), was based on and adapted from the methodology described by Villas Boas [11]; the labeling conditions studied include reaction temperature, time and buffer pH (0.5M sodium ascorbate). In each labeling, 20 µm of PSMA-I&T were used, and different lutetium-177 activities, to evaluate the effect of the peptide:lutetium molar ratio.

2.2 Quality Control

The radiochemical purity (% RP) was performed by HPLC (Shimadzu, USA), adapted to the method described by Villas Boas [11], with a gradient of A= H_2O/0.1 % TFA and B=ACN/0.1 % TFA (0-8 min 24% B; 9-18 min 60% B); flow 0,6 mL/min, RP18 column 5µm 4.6 x 150 mm, 24ºC and injection volume of 100 µL.
Radiochemical Purity by Thin Layer Chromatography was performed using silica gel 60 (TLC-SG) coated aluminum strips as stationary phase and 0.1M sodium citrate buffer, pH 5,0 as mobile phase and the radioactivity was detected with Gamma Counter (Packard, USA). The obtained results were confronted with the acceptance criterion ≥ 97%.

The stability of the preparations was determined after 24 and 48 hours of radiolabeling, stored under freezing, and at least three experiments were performed for each experimental condition (N=3).

3. Results and Discussion

The experimental design adopted made it possible to analyze PSMA-I&T radiolabeling under different conditions. A direct relationship was observed between the %RP and the peptide:lutetium molar ratio, with a significant drop in the %RP from the molar ratio 6.8 to 2.8.

Table I presents the %RP results in the study of pH buffer variation. No significant differences were observed in the %RP for time zero, however, increasing the pH buffer, the %RP decreased after 48 hours, despite still meeting the acceptance criteria for the test (≥ 97%). Analyzed from the PRISMA® by ANOVA and Tukey's test, there is no significant difference between the pH at 48h (P= 0.4793)

Table I: Results of the variation of the pH buffer (0.5M sodium ascorbate) in the %RP of ^{177}Lu-PSMA-I&T

		% Radiochemical Purity (main peak in HPLC)		
Number of experiments	pH	0	24h	48h
3	4.4	99.9 ± 0.1	99.6 ± 0.3	99.2 ± 0.3
6	4.7	99.4 ± 0.3	99.2 ± 0.2	98.8 ± 1.2
3	5.0	99.4 ± 0.9	98.9 ± 1.1	98.3 ± 1.3

The evaluation of radiochemical purity by CCD aimed to determine the percentage of colloidal lutetium, wich in all labeling pH conditions was lower than 1.6 % .

Figures 1 and 2 present the results of the study of influence of temperature and reaction time, respectively, in the %RP (main peak in HPLC) of the preparations.

More consistent results and with smaller standard deviations were obtained with heating to 95 ºC. Analyzed by ANOVA test, there is no significant differences of temperatures for any time of stability, interaction P=0.8915

Regarding reaction time, no significant differences were observed for 20 and 30 minutes, ANOVA P= 0,0895. However, for 40 minutes the difference is significant (ANOVA test, P= 0,0331) and this reaction time impacted slightly the stability of the product, despite all results obtained met the acceptance criteria for the radiochemical purity test.

Also in time and temperature variation studies, the percentage of free lutetium, analyzed by CCD, did not exceed 3,0%, for temperature and time in all conditions studied.

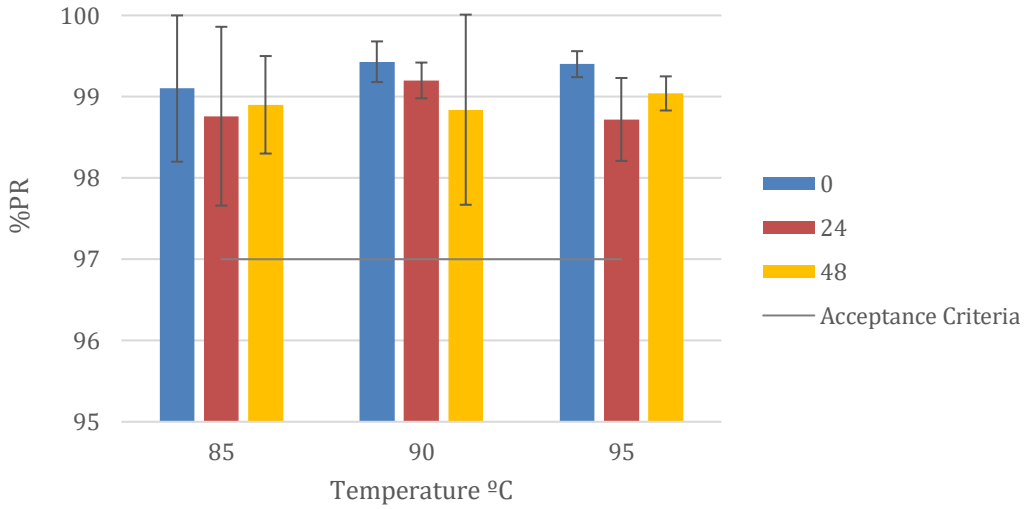

Figure 1: Result of variation in the reaction temperature in the %RP (main peak in HPLC) of ^{177}Lu-PSMA-I&T

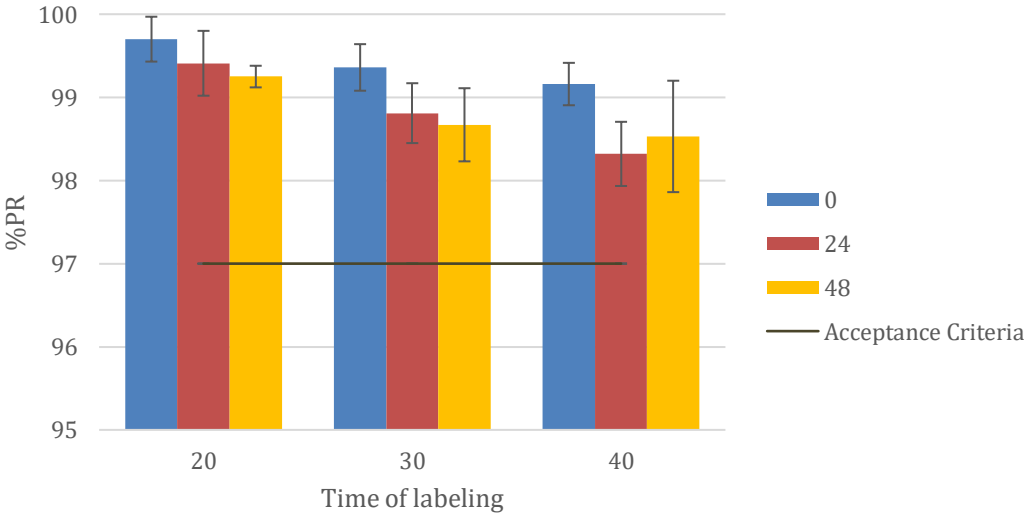

Figure 2: Results of variation in the reaction time in the %RP (main peak in HPLC) of ^{177}Lu-PSMA-I&T

4. Conclusions

With this work it was possible to define the best condition for radiolabeling PSMA I&T with ^{177}Lu.
The next steps of this study include using multifactorial variation to determine the workspace of the radiolabeling step and evaluate the ideal labeling condition to prepare therapeutic doses.

Acknowledgements

This project was carried with federal resources and support from the Institute of Energy and Nuclear Research IPEN / CNEN Brazil. The authors would like to thank the entire staff of the Radiopharmacy Center, for the valuable contribution.

References

[1] "Global Cancer Observatory" https://gco.iarc.fr (2023)

[2] Morbeck, I. A. P. et. al., *Diretrizes Oncologicas - Câncer de Prostata* - 3º edição, São Paulo/ Brasil, (2019).

[3] Chatalic, K. LS. Et. al., *Towards Personalized Treatment of Prostate Cancer: PSMA I&T, a Promising Prostate-Specific Membrane Antigen*, Targeted Theranostic Agent Theranostics, Vol. 6, Issue 6 (2016).

[4] Baum, R.P. et. al., 177Lu-Labeled Prostate-Specific Membrane Antigen Radioligand Therapy of Metastatic Castration-Resistant Prostate Cancer: Safety and Efficacy, *Journal of Nuclear Medicine*, vol. 57, pp. 1006–1013 (2016).

[5] Weineisen, M et. al., 68Ga- and 177Lu-Labeled PSMA I&T: Optimization of a PSMA-Targeted Theranostic Concept and First Proof-of-Concept Human Studies, *Journal of Nuclear Medicine,* vol. 56, pp.1169–1176 (2015).

[6] Vyas M., et al, Stability matters: Radiochemical Stability of Therapeutic Radiopharmaceutical 177Lu-PSMA I&T., *Journal of nuclear Medicine Technology,* vol, 50, pp, 244-247 (2023)

[7] Malik, N. et al., Radiofluorination of PSMA-HBED via AI(18)F(2+) Chelation and Biological Evaluations In Vitro. *Molecular Imaging and Biology*, vol.17, pp. 777-785 (2015).

[8] Ruangma A., et al., PSMA for Pet imaging of prostate cancer. *The Bangkok medical Journal*, vol 14, pp. 95-95 (2019)

[9] Di lorio et al., Production and Quality Control of [177Lu]Lu-PSMA-I&T: *Development of an Investigational Medicinal Product Dossier for Clinical Trials. Molecules,* vol.27, pp.4143 (2022)

[10] Chakraborty S., et al., Prospects of medium specific activity 177Lu in targeted therapy of prostate cancer using 177Lu-labeled PSMA inhibitor. *Journal of Labelled Componds and Radiopharmaceutical,.*vol, 59, pp 364-371 (2016)

[11] Boas, C. A. W. V., et al, In vitro and in vivo response of PSMA-617 radiolabeled with CA and NCA lutetium-177, *Applied Radiation and Isotopes,* vol 180, pp 1-7 (2022).