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Kastrasyona Dirençli Prostat Kanseri Tedavisinde Kullanılan 177LuPSMA-I&T'nin İnvitro Stabilitesinin İncelenmesi

Investigation of The Stability of 177LuPSMA-I&T Prostate-specific Membrane Antigen İnhibitor Used in The Treatment of Castration Resistant Prostate Cancer

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ÖZET

Amaç: Prostat spesifik membran antijeni (PSMA), klatrin kaplı veziküller yoluyla hücreye bağlanır ve ardından endositoz yoluyla hücre içine girer. Bu özelliği nedeniyle, PSMA prostat kanseri görüntülemede Galyum-68 (68Ga) ve tedavide Lutesyum-177 (177Lu) ile işaretlenebilen mükemmel bir moleküldür. Çalışmanın amacı, PSMA-I&T'nin 177Lu ile işaretlenmiş radyofarmasötiğinin (177LuPSMA-I&T) serum fizyolojik içerisinde in-vitro stabilitesini incelemektir.
Materyal-Metod: PSMA-I&T'nin 177Lu ile işaretlemesi otomatik sentez modulünde gerçekleştirildi. İşaretlenmiş 200mCi (7,4 GBq) 177LuPSMA-I&T 37oC'de 72 saat inkübasyona bırakıldı. 72. saate kadar belirli aralıklarla alınan örnekler radyo-yüksek performanslı sıvı kromatografisi (RP-HPLC) ile analiz edildi.
Bulgular: 177LuPSMA-I&T'nin radyokimyasal verimi >% 99 bulundu. PSMA-I&T ile hazırlanan 177LuPSMA-I&T'nin ilk 6 saate kadar stabil kaldığı, 24. saatte ise stabilitesinde %3,3 oranında bozunumun başladığı, 48. saatten sonra ise kullanıma uygun olmadığı belirlendi.
Sonuç: 177LuPSMA-I&T radyofarmasötiğinin ilk 6.saatteki stabilitesi nedeniyle tedavide kullanımının güvenli olduğu, gerektiğinde etiketlemeden sonraki 48 saate kadar kullanılabileceği sonucuna varılmıştır.

Anahtar Kelimeler: Prostata spesifik membran antijeni, PSMA, 177LuPSMA-I&T, kastrasyona dirençli prostat kanseri, radyonüklid tedavisi.

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ABSTRACT

Objective: Internalization of the prostate-specific membrane antigen (PSMA) by ligand binding through clathrin-coated vesicle followed by endocytosis provides it an excellent target in prostate cancer imaging Gallium-68 (68Ga) and treatment Lutetium-177 (177Lu). The purpose of this research is to examine the in-vitro stability of the radiopharmaceutical (177LuPSMA-I&T) labeled with 177Lu of PSMA-I&T in saline.
Material-Method: Labeling of PSMA-I&T with 177Lu was carried out in the automated synthesis module. The labeled 200mCi (7.4 GBq) 177LuPSMA-I&T was incubated at 37oC for 72 hours. Samples taken at regular intervals up to 72 hours were analyzed by radio-high performance liquid chromatography (RP-HPLC). Results: The radiochemical efficiency of 177LuPSMA-I&T was > 99%. We determined that 177LuPSMA-I&T prepared with PSMA-I&T remained stable up to 6 hours, and deteriorated by 3.1% at the 24th hour. Conclusions: With the easy and fast labeled 177LuPSMA-I&T radiopharmaceutical is safe to use in treatment due to its stability in the first 6 hours.

Keywords: Prostate-specific membrane antigen, PSMA, 177LuPSMA-I&T, castration-resistant prostate cancer, radionuclide therapy.

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1. INTRODUCTION

In prostate cancer, approximately 15% of the cases are malignancies that progress to metastatic castration-resistant prostate cancer (mCRPC) and approximately 90% of them occur with bone metastasis (1, 2). Survival times in mCRPC with the effect of newly developed drugs (e.g. Enzalutamide, abiraterone) it increases with each passing year. Lutetium-177 $(^{177}_{71})Lu$ is approval in labelled form by the Food and Drug Administration (FDA, 2018) in 2018.

PSMA labeled with 177Lu is used in the diagnosis of PC recurrence and metastases and in the treatment of advanced stage mCRPC. The physical half-life of 177Lu is 6.647 days. 177Lu emits short range β rays in soft tissue (average 0.23mm, max.1.7mm) and γ rays. The only study in the literature that theoretically deals with the production of a 177Lu radioisotope by sending charged particles onto a target was conducted by Kambali (3). In addition, the 177Lu radioisotope is generally produced by the 176Yb (n, γ) 177Yb → 177Lu reaction in nuclear reactors and the 176Yb (d, n) reaction to produce a 177Lu radioisotope in a cyclotron (4,5). Lutetium is a rare earth element with atomic number 71 (6). The possibilities of using 177Lu in radiopharmaceutical grades used in radionuclide therapy are still under investigation. Among the studies that are in the R&D stage, only 177Lu-DOTA-TATE radiopharmaceuticals have been approved for clinical use (7). Suitable chelates for use in the 177Lu radiolabeling process are linked with synthesized peptides. In chelate selection; it should be noted that the radiometal-chelate complex has a high stability in the biological environment that cannot be separated from each other and during the formation of the chelate-peptide complex, the chelating property should not be forgotten that it should not be lost. Chelates to be preferred should be able to form complex with various elements with appropriate physical properties and be commercially available in reactive form (7,8). Common pollution available in the reagents (Fe, Al, Mn, Ni, Cu, etc.) compete with 177Lu for the chelating agent (9,10).

Recently, in this group of radiopharmaceuticals, the use of 177LuPSMA treatments was started (11,12). PSMA is a transmembrane protein consisting of 750 amino acid chains encoded on chromosome 11 and having both intracellular and extracellular domains (13,14). There are several different PSMA peptides labeled with 177Lu in clinical use as therapeutic agents in men with mCRPC. PSMA-617, PSMA-11 and EuK-Sub-kf-(3-iodo-y-) DOTAGA (PSMA-I&T) are the most preferred PSMA ligands in PSMA-targeted radionuclide therapy (15). In a series of studies with the PSMA-I & T peptide, this agent has been reported to be effective. The use of PSMA-I&T, which has become widespread with the use of different derivatives of PSMA, has drawn attention to this peptide.

The basic principle of treatment with radionuclides is the treatment with the maximum dose that does not cause toxicity to the patient. The binding ratio and stability of the prepared radionuclide must be examined through quality control before starting the treatment. In this study, examined the in vitro stability of 177Lu PSMA-I&T in saline.

Table 1. RAD dedector analysis result of the 177LuPSMA-I&T RP-HPLC chromatogram at the fig.1

Reten. Time [min]	Area [mV.s]	Area [%]	Height [mV]	Height [%]
5.251	3166.786	100.0	359.019	100.0

2. MATERIAL-METHOD

Reagents

Non-radioactive PSMA-I&T, used for the present study, and casette equipment for the synthesis of 177Lu were obtained from ABX D-01454 Radeberg (Germany). The casette components are; sodium ascorbate (13.0±0.6mg), sodium acetate-trihydrat (31.0±1.5 mg), 0.04 M acetic acid solution, sodium chloride / DTPA solution (10.0±0.2 mg). PSMA-I&T peptide was stored at -20 °C. Dilutions of PSMA-I&T were prepared with farmako brand sterile water (1:1). The casette exhibits a disposable casette and is therefore made for single use. [177Lu]$LuCl_3$ used in the present work was obtained LuMark® 177Lu (IDB Holland, Netherlands).

Table 2. RAD dedector analysis result of the 177LuPSMA-I&T RP-HPLC chromatogram at the fig.2

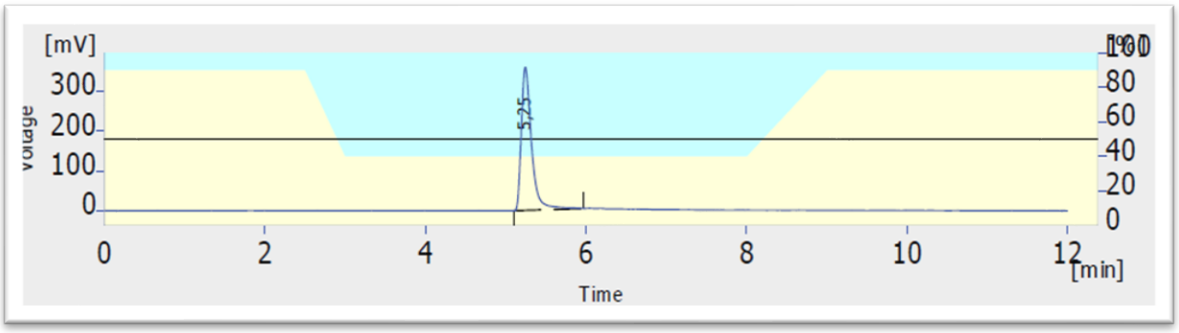
Components	Reten. Time [min]	Area [mV.s]	Area [%]	Height [mV]	Height [%]
A	5.245	3164.986	100.0	358.019	100.0
B	1.453	69.579	3.1	7.399	3.0
	5.255	2167.822	96.9	239.063	97.0
C	1.452	89.112	3.3	10.186	3.2
	5.233	2648.778	96.7	305.536	96.8
D	1.481	627.109	26.7	48.442	27.4
	5.240	1723.081	73.3	28.153	72.6

The radio-high performance liquid chromatography (RP-HPLC) system consists of an Agillent1100 quaternary pump with a degasser (Germany), Welch Ultisil XB-C18 column (3.0xmm, 3 µm) column; SCI8120 UV/Vis detector (Germany). DataApex Clarity programme (Raytest, Germany).

System Description and Synthesis of ^{177}LuPSMA-I&T and quality control

PSMA-I&T radioactive labeling was performed in the automatic synthesis module. Calculated amount of peptide, sodium ascorbate buffer, pH 4.5 with 0.04M acedic acid solution was labeled at 90°C for 15 minutes. After cooling the reaction flask to room temperature, 7±0.2 mL (2µg) of sterile DTPA solution was added. The final product is passed through the 0.22 µm filter syringe and collected in the final vial. Radiochemical purity was determined by radio-high performance liquid chromatography (RP-HPLC). Under working conditions maintained with a flow rate at 0.6 mL/min an isocratic separation was performed using a mobile phase, including acetonitrile (30%) and trifluoroacetic acid (TFA, 0.1%) in water at pH 4.5. The samples were monitored at 220 nm with a UV detector and radio detector to determine the binding rate and determine the impurities.

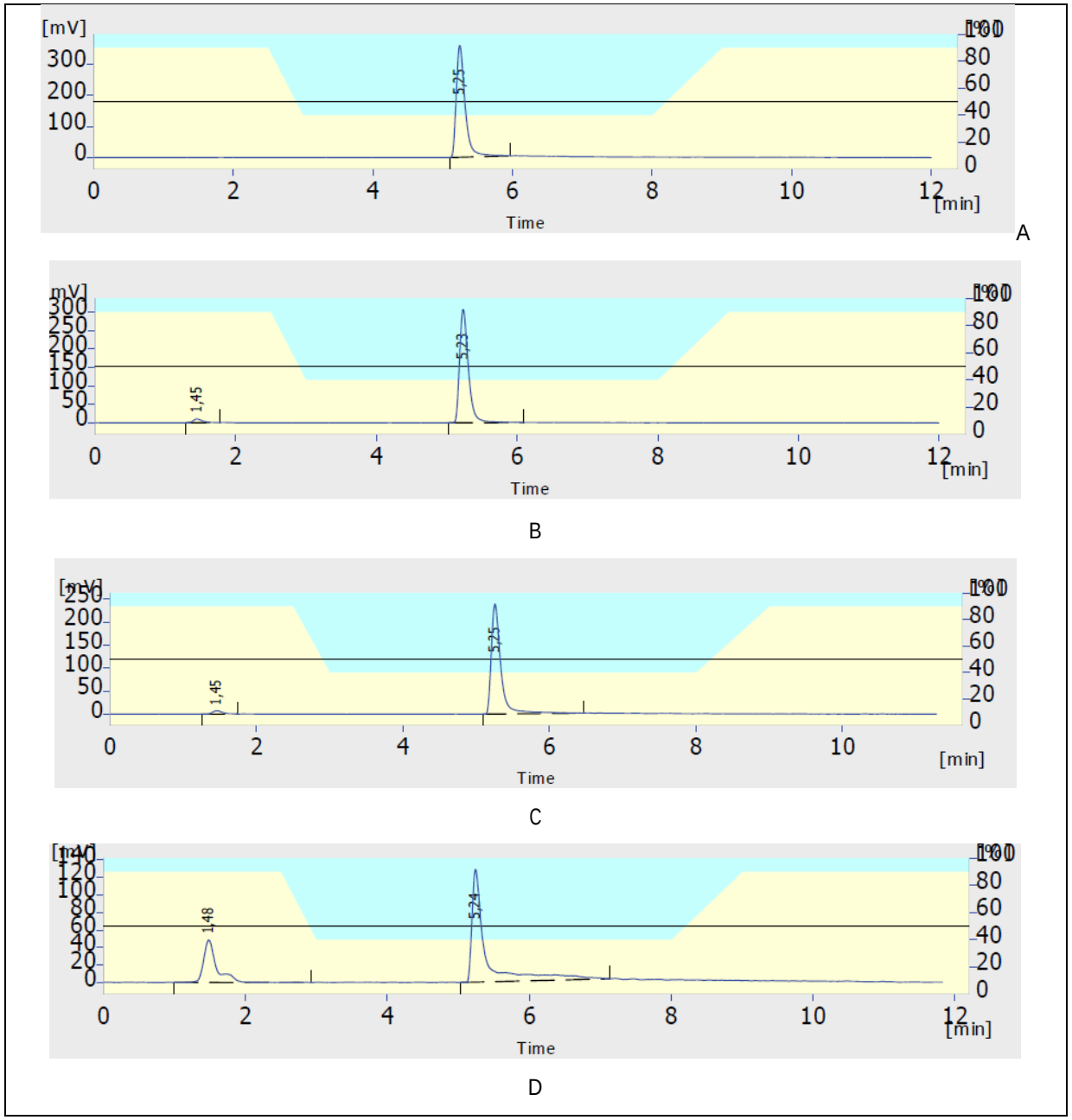
Figure 1. RP-HPLC chromatogram of 177LuPSMA-I&T labeled



Stability of ^{177}Lu-PSMA I&T

Stability studies were performed in DTPA solution in duplicate. Time intervals were adjusted according to the physical half-life of the radionuclide (6, 24, 48, 72 hours).

Figure 2. RP-HPLC chromatograms of 177LuPSMA-I&T (the RP-HPLC elution time of radioligand is in between 4.3-5.3 min) in 200 mCi patient dose incubated in saline at 37 °C A) at 6 h B) at 24h C) at 48h D) at 72h



The prepared patient dose of 177LuPSMA-I&T (200 mCi, 7.4 GBq) was incubated in saline for up to 72 hours at 37 °C. At the specified time intervals incubation solution sample was injected to reverse phase (RP)-HPLC for assess the in vitro stability of the patient dose for up to 72 hours.

3. RESULTS

Synthesis and quality control studies were conducted in a radiopharmaceutical laboratory at Department of Nuclear Medicine, in University Hospital. All reagents used are to analytical purity and the synthesis process is carried out under GMP conditions. Therefore, no additional quality control is required for method validation. The automated synthesis was performed within 32 min. The pH of the final product was determined to be in between 6 and 7. The radiochemical yield of 177LuPSMA-I&T was > 99% by RP-HPLC (fig1, table 1). Samples taken at the 6th, 24th, 48th and 72th hours after the preparation of the

radiopharmaceutical were analyzed by RP-HPLC (fig.2, table2). All experiments were performed in triplicate.
The radio-labeling procedure of 177LuPSMA-I&T is easy and provides consistent high radio-labeling efficiency. Radiochemical purity was over 99%. The radiopharmaceutical, which remained stable in saline up to the 6th hour after radiolabeling, appears to begin to separate PSMA I&T after the 6th hour.

4. DISCUSSION

Similar studies were conducted with 177LuPSMA-617, and they reported that the radiopharmaceutical prepared up to 48 hours remained stable (16). Stability results reported refer to the degradation of the radionuclide-labeled peptide into "free" protein (17). For the analysis of radiolabeled peptides, RP-HPLC is seen as a suitable method to identify all potential degradation products and metabolites (18).
In general, since the labeling of radiopeptides is quantitative, it must be done with great care considering the high assay variability in in vitro stability assays. Different incubation times may be indicative of instability and should be interpreted with caution as binding of different amounts of peptide derivatives may also be responsible for in vitro degradation.

5. CONCLUSION

177LuPSMA-617 radiopharmaceutical is expensive and difficult to obtain, labeling 177LuPSMA-I&T more attractive for treatment. There are studies that 177LuPSMA-I&T treatment is a safe method in the treatment of mCRPC patients (8,9,12,13). 177LuPSMA-I&T is easy to radiolabel and is a stable compound in vitro up to the first six hours post-production. It is considered in necessary cases that 177LuPSMA-I&T can be used for up to 48 hours with a 3.3% decay. As a result, while providing the necessary time for the transport of 177LuPSMA-I&T radiopharmaceutical prepared with PSMA-I&T from the laboratory to the service, it is recommended to use the labeled radiopharmaceutical for up to 6 hours.

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